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SECTION MEETINGS

DISTRICT OF COLUMBIA	
National Institute of Health, Washington	November 23, 1937
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MISSOURI	
Washington University	November 10, 1937
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WESTERN NEW YORK	
University of Buffalo Medical School	December 11, 1937

9599

Amino-acid Content of the Blood Following Intravenous Injection of Hydrolyzed Casein.

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The ability of the body to utilize amino-acids injected intravenously in the rapid regeneration of serum protein was indicated in a recent paper in the Proceedings.[1] The importance of parenteral administrations of nitrogenous nourishment to patients unable to take food by mouth is obvious. Previous experiments on the intravenous injection of a mixture of amino-acids have been reported by Van Slyke and Meyer,[2] by Henriques and Anderson,[3] and others. The observations hereby recorded concern the disappearance rate of amino-acids injected intravenously at two different rates. They were made on dogs fasted for 48 to 72 hours, but allowed water *ad lib.* In each experiment the volume of the amino-acid solution injected equaled 3.5% of the body weight of the dog and consisted of hydrolyzed casein, 5%, in 5% glucose-Ringer's solution. The hydrolyzed casein was soluble in water, gave no precipitate with tungstic acid, and was not anaphylactogenic for guinea pigs The results of the first group of experiments are represented in Fig. 1; the solution was injected slowly, *i. e.*, at the rate of 0.2 cc. per kilo of dog per minute. These animals had all been bled 3.5% of their body weight and the same volume of Ringer's solution replaced ½ hr. before the injection of amino-acid solution was started. Thus, a 10-kg. dog received 350 cc. during a period of 175 minutes. Samples of jugular blood were taken immediately at the conclusion of the injection, and at 15- to 75-minute intervals. In the second group of experiments, represented in Fig. 2, 3.5% of the body weight of the dog was bled; the same volume of 5% amino-acids in 5% glucose-Ringer's solution was then immediately injected in one massive dose. A sample of jugular blood was taken immediately after replacement, and at intervals up to 3 hours later. Few symptoms followed the injection in each group and all animals survived the procedure without evidence of any toxic effect. Control samples of blood from the intact fasting dog revealed a relatively uniform content of amino-acids. Thus, in 10 dogs the following values were obtained (the numbers represent mg. % of amino-acid nitrogen): 5.4; 4.1; 5.1; 5.6; 4.6; 6.2; 5.9; 6.7; 5.4; 5.0—which average 5.3. The Folin colorimetric method, as described by Hawk and Bergeim,[4] was used for the determination of amino-acid nitrogen. Excellent checks were obtained within the normal range. The higher values here reported are probably not so accurate, but since inferences are drawn from relative, not absolute changes, such errors are not significant. The determinations were made on serum after centrifugalization of the clotted blood. The amino-acid solution was prepared from casein by acid hydrolysis; it was generously supplied by Mead, Johnson and Company. In most of the experi-

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[1] Elman, R., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 867.

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[2] Van Slyke, D. D., and Meyer, G. M., *J. Biol. Chem.*, 1913, **16**, 197.

[3] Henriques, V., and Andersen, A. C., *Z. f. Physiol. Chem.*, 1913, **88**, 357.

[4] Hawk, P. B., and Bergeim, O., *Practical Physiological Chemistry*, Tenth Ed., 1931, Blakiston, Philadelphia, p. 427.

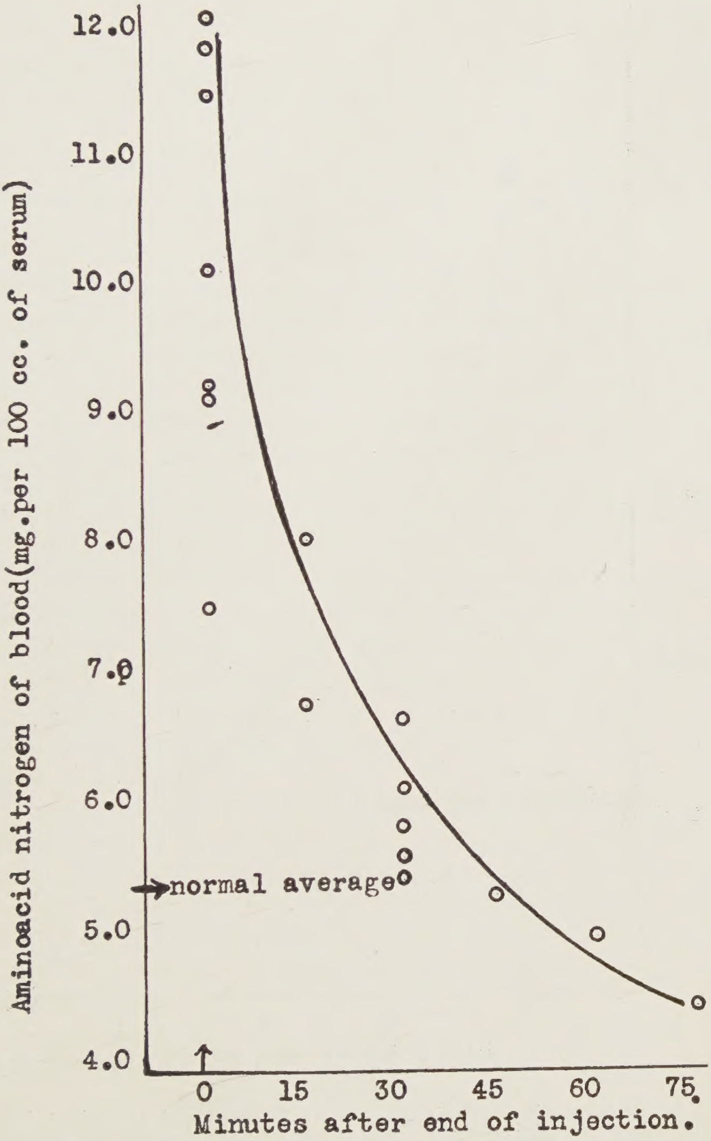

Fig. 1.

Rise and fall of amino-acids in the blood after a slow injection. After bleeding and replacement with Ringer's solution, the amino-acid solution (3.5% of the body weight) was injected intravenously at the rate of 0.2 cc. per minute per kg. of body weight. Six dogs.

ments here reported, 2% tryptophane was added to the product before making up the solution.

Conclusion. After a relatively slow, intravenous injection of 5%

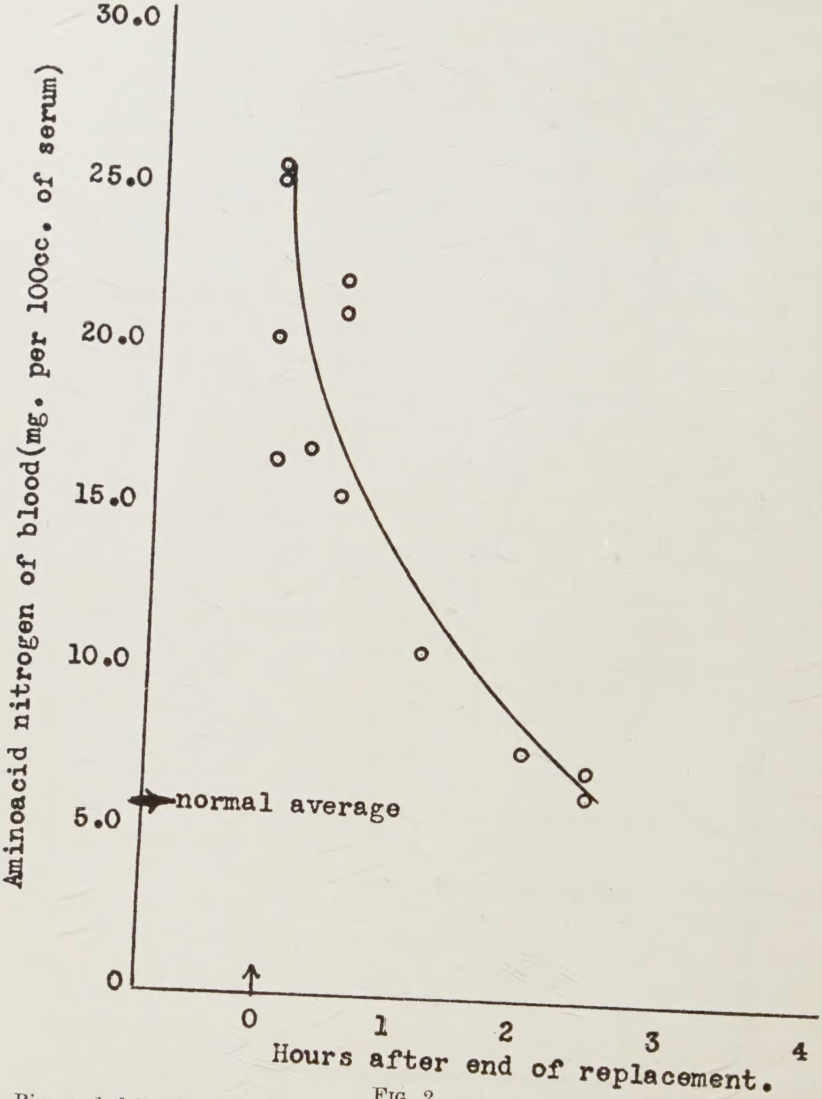

FIG. 2.

Rise and fall of amino-acids in the blood after a rapid (massive) injection. The same amount of amino-acid solution (3.5% of body weight) was given immediately after bleeding the same amount. Four dogs.

amino-acid solution, the concentration of amino-acids in the serum rises, but falls rapidly within 30 minutes to the normal level. After a massive intravenous injection, the value rises much higher but returns to normal within 2½ hours. The range of the amino-acid nitrogen concentration of the serum of 10 fasting dogs extends from 4.1 to 6.7, with the average at 5.3 mg. %.

9600

Prevention of Cholesterol Arteriosclerosis in the Rabbit by Use of Pancreatic Extract (Lipocaic).

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Dragstedt, Van Prohaska and Harms[1] reported observations on a substance present in pancreas which permits survival and prevents liver changes in depancreatized dogs. To this substance they gave the name "Lipocaic". The interesting effects of this substance on lipoid metabolism led us to investigate its effect on the atherosclerosis which can be produced in rabbits by feeding cholesterol and foods high in cholesterol content. This experimental vascular change in rabbits has been studied particularly by Anitschkow,[2] Bailey,[3] Clarkson,[4] Dominguez,[5] and Duff.[6]

Lipocaic was prepared according to the method of Dragstedt, Van Prohaska and Harms.[1] The procedure involves the extraction of fresh pancreas with 60% ethyl alcohol. The alcohol extract is then evaporated at a temperature less than 40°C. The residue is extracted with sulphuric ether. The fat-free extract dissolved in water was so diluted that 5 cc. contained the extract of 50 gm. of pancreas.

In this study, the diet fed to all the rabbits consisted of a standard ration of oats, Purina rabbit chow and green vegetables. Each rabbit receiving the high cholesterol diet was given each morning one 50 cc. beaker of this chow containing 0.5 gm. cholesterol and one-half egg yolk. No difficulty was experienced in making the animals eat this mixture. After eating this, they were given additional food.

In the case of the hormone, the preparation of which was described above, 5.0 cc. of the concentrated watery substance containing the extract obtained from 50 gm. of pancreas was added to a 50 cc. beaker of Purina rabbit chow. Each rabbit receiving the hormone was given one of these beakers of chow daily.

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[1] Dragstedt, L. R., Van Prohaska, J., and Harms, H. P., *Am. J. Physiol.*, 1936, **117**, 175.

[2] Anitschkow, N., *Beit. z. Path. Anat. u. z. Allg. Path.*, 1913, **56**, 379.

[3] Bailey, C. H., *J. Exp. Med.*, 1916, **23**, 69.

[4] Clarkson, S., and Newburgh, L. H., *J. Exp. Med.*, 1926, **43**, 595.

[5] Dominguez, R. J., *Exp. Med.*, 1927, **46**, 463.

[6] Duff, G. L., *Arch. Path.*, 1935, **20**, 81, 259.

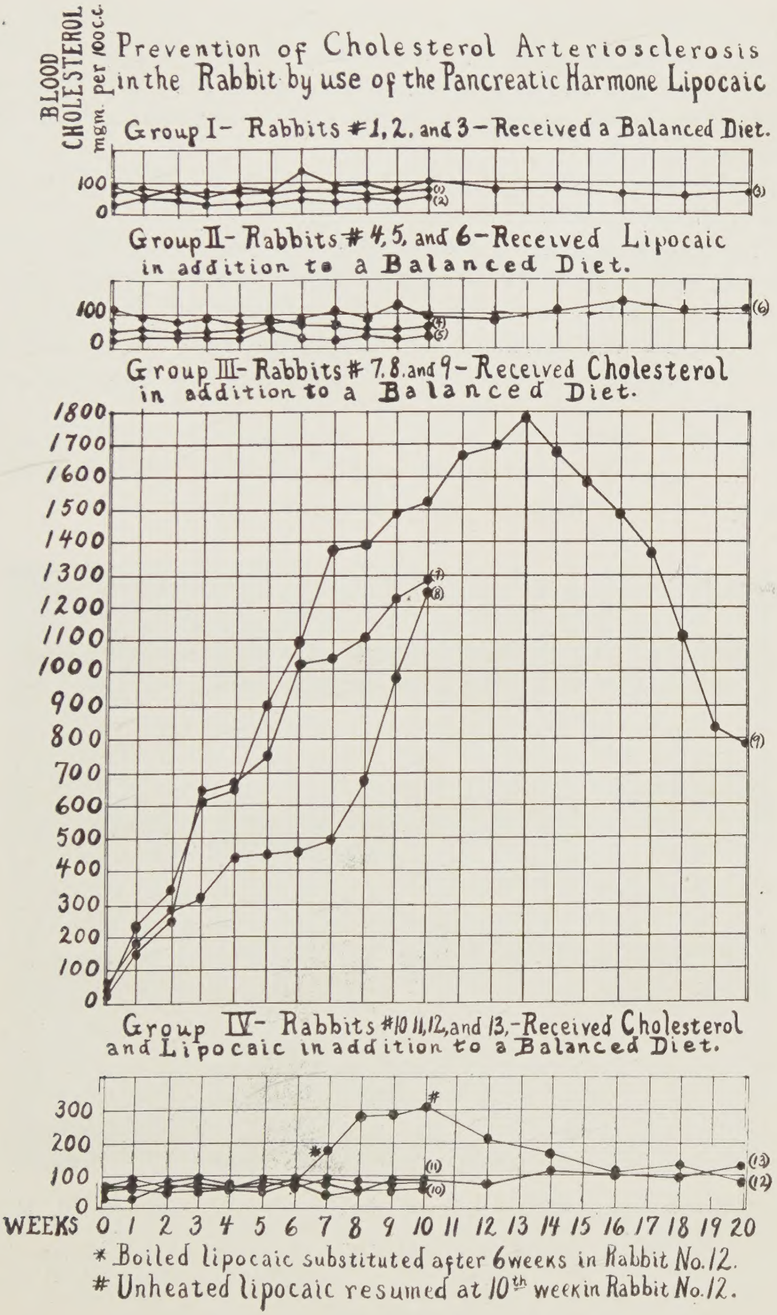
TABLE I.

Prevention of Cholesterol Arteriosclerosis in the Rabbit by Use of the Pancreatic Hormone Lipocaic.

Blood Cholesterol, mg. per 100 cc.

Rabbit No.	Group I Received Balanced Diet			Group II Received Lipocaic and Balanced Diet			Group III Received High Cholesterol and Balanced Diet			Group IV Received High Cholesterol Balanced Diet and Lipocaic				Group V Rabbit No. 14 Received Balanced Diet, Cholesterol, and Lipocaic Heated to 90° C.	Group V Rabbit No. 15 Received Balanced Diet, Cholesterol and Lipocaic Heated to 65° C.	Group VI Rabbit No. 16 Received Balanced Diet and Cholesterol for Ten Weeks, then Cholesterol Was Discontinued
Weeks	1	2	3	4	5	6	7	8	9	10	11	12	13			
0	74	25	87	80	21	109	33	50	47	31	72	51	64	25	48	48
1	84	56	64	93	34	97	152	192	235	21	82	58	56	44	43	222
2	76	56	83	87	33	87	265	270	334	63	59	43	74	37	58	309
3	77	37	55	70	38	94	640	305	606	56	68	51	91	847	292	334
4	72	38	78	98	33	74	670	441	658	50	55	59	62	889	376	494
5	67	36	69	94	69	73	740	445	904	47	81	83	67	1107	455	633
6	76	47	131	71	30	77	1017	454	1085	75	64	79*	83	1187	474	708
7	64	34	94	68	21	104	1026	494	1380	37	93	177	76	1474	555	808
8	56	51	97	49	43	91	1105	684	1389	51	89	278	56	1389	546	952
9	60	39	73	45	37	119	1220	989	1480	56	79	281	92	1333	591	1085
10	71	47	101	55	40	96	1290	1250	1510	58	92	303†	89	1275	658	1135
11									1666							
12			82			94			1680			204	77			993
13									1780							
14			85			101			1677			167	111			705
15									1594							
16			77			123			1481			108	104			658
17									1367							
18			68			108			1111			121	99			593
19									808							
20			75			112			794			81	119			581
Autopsy Findings																
Gross Atherosclerosis of Aorta	—	—	—	—	—	—	++++	+++	++++++	—	—	—	—	++++	++ to +++	++++++
Microscopic Atherosclerosis of Aorta	—	—	—	—	—	—	++++	+++	++++++	—	—	—	—	++++	++ to +++	++++++

*Boiled hormone substituted after this cholesterol determination. †Unheated hormone treatment resumed.



* Boiled lipocaic substituted after 6 weeks in Rabbit No. 12.

# Unheated lipocaic resumed at 10th week in Rabbit No. 12.

CHART 1.

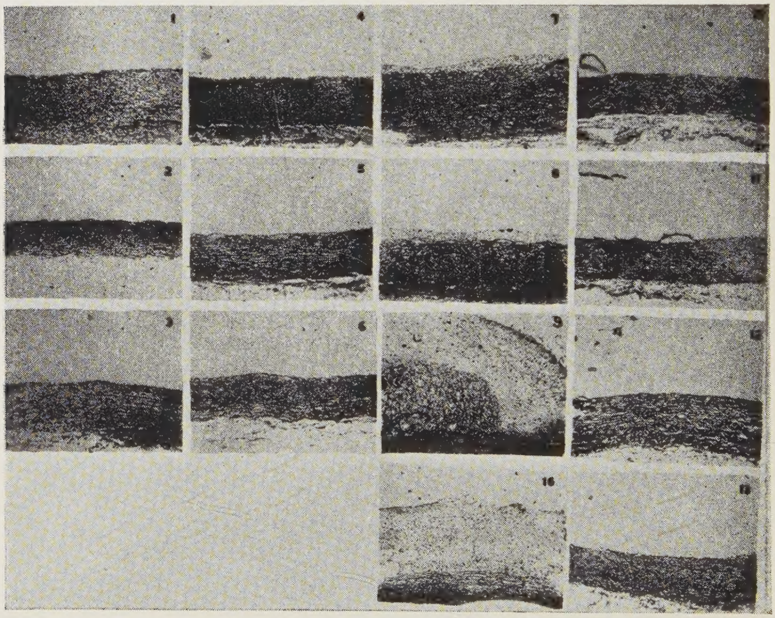
All animals used in this experiment were approximately 6 months of age at the beginning of the experiment. Rabbits Nos. 3, 6, and 13 were from one litter, and Nos. 11, 12, and 16 from another litter.

The procedures carried out in the six groups of rabbits are described in Table I.

Weekly determinations of blood cholesterol were done according to a modification of the method of Bloor.[7]

The feeding experiment continued for 10 weeks after which Rabbits 1, 2, 4, 5, 7, 8, 10, 11, 14, and 15 were sacrificed and an autopsy done. Rabbits 3, 6, 9, 12, 13, and 16 were observed for a period of 20 weeks and then sacrificed and an autopsy done. Gross and microscopic studies were made of the vascular system and organs of all animals.

The effects of the administration of lipocaic are evident both in the changes in blood cholesterol and in the gross and microscopic appearance of the aorta of the rabbits at an autopsy.



Rabbits 1, 2, and 3 received balanced diet.

Rabbits 4, 5, and 6 received balanced diet and lipocaic.

Rabbits 7, 8, 9, and 16 received balanced diet and cholesterol.

Rabbits, 10, 11, 12, and 13 received balanced diet, cholesterol and lipocaic.

Chart 2.

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[7] Bloor, W. R., *Quant. Clin. Chem.*, 1932, **2**, 884.

The results are shown in Table I. The blood cholesterol changes in the first 4 groups of animals are graphically illustrated in Chart 1. The microscopic appearance of the aorta of the experimental animals 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, and 16 is shown in Chart 2.

Lipocaic does not affect the level of blood cholesterol in normal animals. It prevents the rise in blood cholesterol and the development of atherosclerosis in the aorta which otherwise regularly occurs in rabbits fed a high cholesterol diet. Heated hormone is ineffective.

Studies are in progress concerning the use of lipocaic in the treatment of atherosclerosis in rabbits in whom the condition has been allowed to develop. The resemblance of cholesterol atherosclerosis of rabbits to the pathology of sclerotic vascular disease in man suggests the possibility of the use of this pancreatic extract in the prevention and treatment of human atherosclerosis.

9601 P

Synaptic Connections in the Celiac Ganglia.

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The distribution of axons effecting synaptic connections in the celiac ganglia and their modes of termination have been studied in silver preparations of human material and material obtained from normal and experimental animals (cats). The synapses observed involve (1) pericellular and peridendritic nests, (2) arborizations of axons in dendritic glomeruli, and (3) axon terminations in dendritic fasciculi. Synapses of these types have been described by de Castro[1] in various autonomic ganglia. The synapses effected by the terminal branches of axons with dendrites in dendritic fasciculi probably are the most common in the celiac ganglia. Every ganglion cell in these ganglia probably is synaptically related to more than one axon.

Bilateral section of the splanchnic and vagus nerves and extirpation of the upper lumbar segments of the sympathetic trunks in cats resulted in degeneration of the great majority of the axons which effect synaptic connections in the celiac ganglia but not all of them. Degeneration of only the vagus nerves did not appreciably alter the

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[1] de Castro, F., 1931, Sympathetic Ganglia; Normal and Pathological. Cytology and Cellular Pathology of the Nervous System (Penfield), Sec. VII.

numbers or distribution of synapses in these ganglia. Section of nerves extending from the celiac ganglia to the stomach and intestine resulted in extensive degeneration of the fibers in the distal portions of the nerves and degeneration of some fibers in the proximal portions. The latter observation supports the assumption that fibers which arise in enteric ganglia join the celiac plexus. The synaptic connections which persist in the celiac ganglia following degeneration of the splanchnic and vagus nerves probably are effected by these fibers. They probably constitute connections through which reflex responses in one segment of the gastro-intestinal canal may be elicited by impulses arising in another segment.

9602

Influence of Cattle Anterior Pituitary Extract on Endochondral Ossification in Young Ovariectomized Guinea Pigs.*

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Continuing our investigations[1] on the growth of cartilage and bone of the young guinea pig it seemed of interest to analyze the rôle of the gonads, to determine whether or not the effect of anterior pituitary is exerted by intermediation of the ovaries, which organs undergo considerable changes after the administration of anterior pituitary preparations (Loeb[2]).

Observations extended over periods of 1, 2, 3, and 4 weeks. In each of the 4 series, 6 animals averaging 140 gm. in weight were used: (a) One served as normal control; (b) one received daily injections of acid extract of cattle anterior pituitary; (c) 2 were ovariectomized; (d) 2 were ovariectomized and subsequently received daily injections of extract.

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* These investigations have been carried out with the aid of a grant from the International Cancer Research Foundation to Dr. Leo Loeb and with the aid of a grant from the Research Committee of the American Medical Association.

[1] Silberberg, M., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1423; Silberberg, M., and Silberberg, R., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 622.

[2] Loeb, L., *Endocrinology,* 1932, **16**, 129; PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1335; Loeb, L., and Hayward, S. J., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 250; Loeb, L., Festschrift dedicated to Robert Tilden Frank, June, 1937, p. 2, press of The C. V. Mosby Company, St. Louis.

Six more animals which died at intermediate stages served as additional material. Altogether 30 guinea pigs were used.

Under ether anesthesia, both ovaries were removed in 16 animals. To 8 of the latter, starting 2 to 3 days after the operation, intraperitoneal injections of 1 cc. freshly prepared acid extract of cattle anterior pituitary were given. The weights of the guinea pigs, which all were kept under the same environmental and nutritional conditions, were taken daily. At the end of the experimental term, *i. e.,* after 9, 16, 23, and 30 days, femur, knee joints, tibiae, ribs, vertebrae and jaws were fixed, incompletely decalcified, and stained, as previously described.

Gross Changes. The ovariectomized animals grew rapidly and gained much weight (Group III), distinctly more than the normal control animals (Group I). Both groups of injected guinea pigs remained smaller and lighter in weight (Groups II and IV).

TABLE I.

Days of Observation	Group I Normal. 4 animals	Group II Injected. 4 animals	Group III Ovariectomized. 10 animals	Group IV Ovariectomized and Injected. 12 animals
1	135	136	140	144
5	155	146	166	149
7	162	148	177	152
9	165	146	179	150
11	170	150	182	152
13	175	152	186	158
15	190	147	200	163
17	190	155	207	163
19	190	155	223	163
21	185	160	237	160
23	205	155	235	167
25	210	165	247	167
27	215	172	262	170
29	225	174	270	167

The table demonstrates the mean weights of the animals of the 4 groups, the vertical columns representing the weights in grams.

We studied microscopically the changes which took place in the upper epiphysis of the tibia.

(a) *Normal Guinea Pigs* (4 animals): The epiphyseal line (Illustration a) is regular and patent; the cartilage cell layers are sharply demarcated, the intercellular tissue being well developed. The cells of the chondrophyte, *i. e.,* of the lateral cartilaginous protuberances, are resting. In completely healthy spring guinea pigs, the first signs of closure of the epiphyseal line were not detected before the animals had reached a weight of about 440 gm.

(b) *Injected Control Guinea Pigs* (4 animals): The results obtained in this group (Illustration b) agree with those previously communicated.[1]

(c) *Ovariectomized Guinea Pigs* (10 animals): One week following ovariectomy, a marked hyperplasia and hypertrophy, in particular of the columnar and vesicular cartilage cells, becomes manifest; the intercellular tissue is diminished in amount. Four weeks after ovariectomy, (Illustration c), the epiphyseal line of the large animals is very wide, wider than at any period of normal growth, and still completely open. The newly grown cartilage cells, then, do

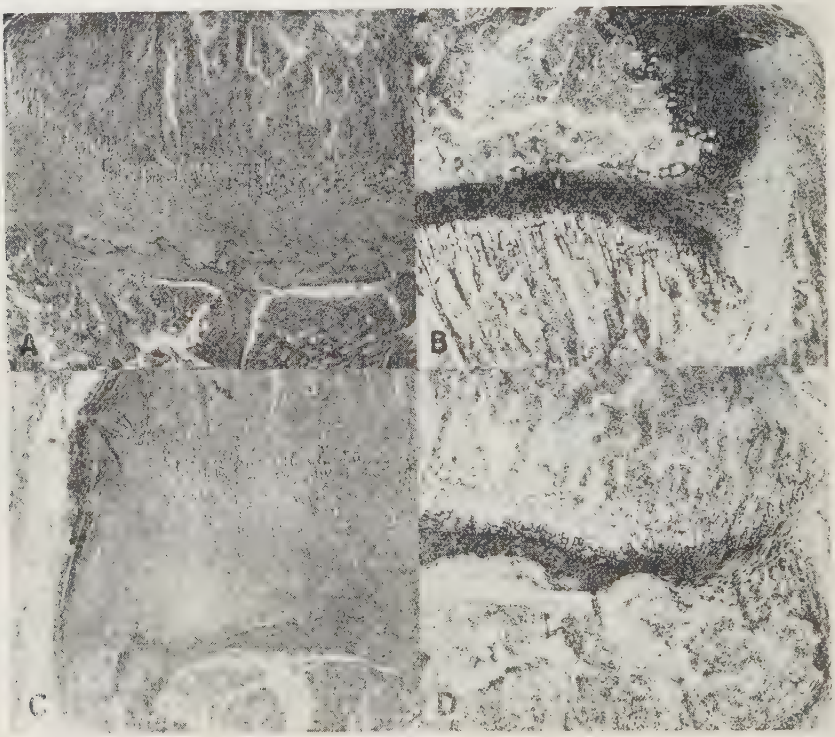

FIG. 1.

Photomicrographs (medium magnification):

The specimens were incompletely decalcified (Pommer's method) and stained with hematoxylin and eosin. The calcified areas appear intensely black.

(a) Open epiphyseal line and chondrophyte of a normal control guinea pig (225 gm.).

(b) Calcified epiphyseal line and hyperplastic chondrophyte after 7 injections of acid extract (weight of guinea pig 148 gm.).

(c) Enlarged, not calcified epiphyseal line of a guinea pig 4 weeks after ovariectomy (weight 280 gm.).

(d) Partly calcified epiphyseal line, hyperplastic and hypertrophic chondrophyte 4 weeks after ovariectomy and 28 injections of acid extract (weight of guinea pig 172 gm.).

not reveal any tendency to calcification, but only slight amounts of calcium deposit are seen in the periphery of the cartilage cells and within the cartilaginous interstitial ground substance, in places where normally much calcification would occur. Also within the chondrophyte, calcification within the growing cartilage is lacking.

(d) *Ovariectomized and Injected Guinea Pigs* (12 animals): The epiphyseal line is somewhat wider than that of the animals which received merely injections of extract, but it is narrower than that of the corresponding normal animals. As early as after 7 injections, the resting, but in particular the columnar and vesicular cartilage becomes hyperplastic. In addition, a marked hypertrophy and subsequent calcification in the cartilaginous cells and the interstitial stroma becomes noticeable; the latter processes representing lines of calcification in the cartilaginous ground substance, however, are evidently less pronounced than in the injected control animals. They differ in degree in accordance with the responsiveness of the animals to the calcifying effect of the extract which may be very accentuated even at earlier stages, but usually becomes gradually more marked and is most pronounced at later stages. After 4 weeks' treatment (Illustration d), in the most advanced cases, strands of hypertrophic blue, more or less calcified cartilage may be observed protruding bud-like into the epiphyseal bone; the epiphyseal line is not yet closed at this period which indicates that the alterations were never so advanced as in the cases of injected non-ovariectomized guinea pigs. In general, at the earlier stages, hyperplastic processes predominate, while later hypertrophy and calcification are more pronounced. The chondrophyte reveals likewise much blue, growing cartilage. In addition, one may detect an ingrowth of the cartilage of the chondrophyte toward the center of the bone and between the zone of the resting cartilage and the bony demarcation of the epiphysis. The histological appearances vary; they depend upon the predominating effective principle and on the duration of the experiment: thus, sometimes the action of ovariectomy, sometimes that of the extract is more accentuated.

Summary. In growing guinea pigs, ovariectomy causes hyperplasia and hypertrophy of the epiphyseal cartilage, whereas its calcification and ossification are not accelerated in the same ratio. Consequently, the closure of the epiphyseal line is delayed, and a relative increase in the size of the individual bone may occur. Whether these changes may lead to gigantism-like, permanent conditions or whether they are only of a temporary nature, which may become compensated at a later stage, is not as yet decided. The above effect of ovariectomy is interfered with by the interaction of acid extract of cattle anterior pituitary, which has the tendency to exert its specific growth-promoting and calcifying influence on the cartilage. If the two factors act simultaneously, a competition between both principles takes place and a combination effect results, the outcome of which determines the course of the endochondral ossification.

9603

Enzymes in Ontogenesis (Orthoptera). III. Activation of Naturally Occurring Enzymes (Tyrosinase).*

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Interest in the enzyme tyrosinase seems largely to have centered around its distribution as well as the nature of its action on various natural and artificial substrates. Its rather wide occurrence in plants seems well established while for animals data are available which would lead one to assume a rather limited or restricted distribution (Graubard and Nelson,[1] v. Euler,[2] Graubard,[3] Raper[4]). Insects appear to be rich in the enzyme (Bodine and Boell[5]).

The present paper treats of results of a study on the growth, activation and substrate relations of tyrosinase as found in the developing egg of the grasshopper, *Melanoplus differentialis*. The advantages in using such biological material have been previously indicated and mention will only be made where departures from usual procedures occur.[5] Enzyme preparations were obtained from centrifuged fractions of egg brei after the following manner. For each set of experiments, 200 eggs of known age were removed from pods and carefully selected for morphological stage of development. They were thoroughly washed in tap water, then sterilized in 70% alcohol, washed three times with sterile distilled water and finally

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* Aided by a grant from the Rockefeller Foundation for research in cellular physiology.

[1] Graubard, Mark, and Nelson, J. M., *J. Biol. Chem.*, 1935, **112**, 135.

[2] v. Euler, H., 1934, *Chemie der Enzyme*, J. F. Bergmann, München.

[3] Graubard, Mark, *J. Genetics*, 1933, **27**, 199.

[4] Raper, H. S., *Physiol. Reviews*, 1928, **8**, 245.

[5] Bodine, J. H., and Boell, E. J., *J. Cell. and Comp. Physiol.*, 1935, **6**, 263.

twice with sterile buffered (phosphate pH 6.8) 0.9% sodium chloride. Excess fluid was allowed to drain from the eggs which were then transferred to a sterile glass mortar and thoroughly ground. Two cc. of buffered 0.9% sodium chloride were added and mixed with the brei. The mixture was then poured into a sterile graduated centrifuge tube. The washing of the mortar with buffered 0.9% sodium chloride was repeated 3 times and the contents of the centrifuge tube brought to volume, 10.5 cc., with buffered 0.9% sodium chloride. This diluted egg brei was then centrifuged for 10 minutes in an International Clinical centrifuge at a speed of 1060 r.p.m. At the end of this period the contents were stratified into 3 definite and clear-cut layers: a thin topmost layer, *A*, of lipoidal and yellow pigment material; a middle, slightly turbid layer, *B*, making up by far the greatest of the three; a dark layer, *C*, about 0.5 cc. in volume and made up of the remains of the membranes, etc., of the eggs. The yellow lipoidal layer was carefully removed by means of filter paper and a capillary pipette attached to an aspirator. The layer *B* was poured into a sterile centrifuge tube leaving *C* behind. Fraction *B*, which was the one employed in the following experiments was then ready for use and as prepared was equivalent to 20 eggs per cc. of solution. All determinations of enzyme concentration were carried out in standard Warburg manometers at 25°C. using tyramine hydrochloride as substrate, essentially as indicated previously.[5]

In preliminary experiments, it was easily demonstrated that the enzyme tyrosinase was largely if not solely confined to the *B* fraction of the above mixture. However, if the *B* fraction is added to the substrate, tyramine HCl, very little activity occurs over long periods of time, indicating the possible existence of an inhibitor or the lack of a naturally occurring activator. By checking back it was soon discovered that the addition of the fraction *A* caused an almost immediate response on the part of the enzyme in the *B* fraction. A detailed study was next made on the question of other possible activators for the enzyme. A large list of compounds was found to work rather favorably in this respect. The list of activators includes the following: chloroform, acetone, ethyl urethane, sodium taurocholate, ether, heat (60°C. for 10 min.), sodium oleate, toluol, thymol, olive oil, etc. Results are presented using only sodium oleate as activator since this compound has been found to function in this respect especially well. In a typical experiment the various constituents of the manometer flasks were made up as follows: 1 cc. of *B*; 0.1 cc. of 5% sodium oleate; 0.1 cc. of 10% potassium hydroxide (center well); 1.6 cc. of buffered (pH 6.8) 0.9% sodium chloride; 0.3 cc. of 0.4% tyramine HCl (in side bulb).

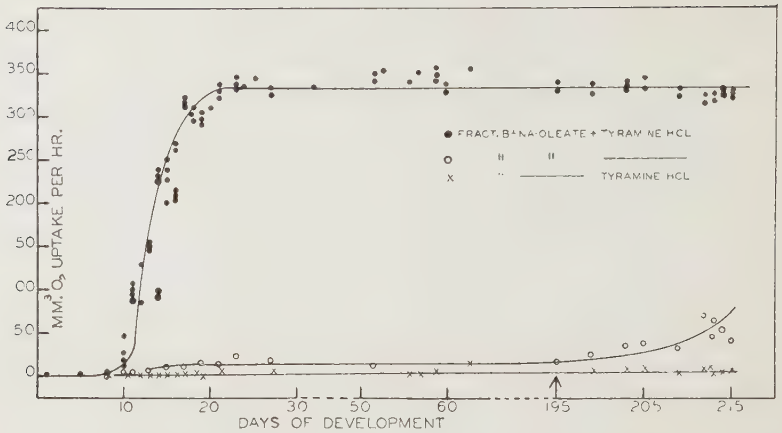

FIG. 1.

Figure shows amount of enzyme (tyrosinase), its relative activity, as well as naturally occurring substrate in the developing egg of the grasshopper *(M. differentialis)* from laying to hatching. Abscissa, indicates days of development at 25°C. Arrow indicates beginning of post-diapause. Ordinate, cubic millimeters of oxygen consumed per hour by enzyme extract B (equivalent to 20 eggs per cc. of extract). Points indicated for individual experiment in most cases, otherwise represent averages.

Results of many experiments have been graphically shown in the accompanying figure. From an inspection of this figure it will be noted that the enzyme in fraction B without the addition of sodium oleate shows practically no activity during the entire course of the development of the egg. Upon addition of sodium oleate, however, marked activity occurs. As a matter of fact the theoretical oxygen uptake in many cases is approximated at the end of an hour. That the total enzyme present has not been used up can be clearly demonstrated upon the addition of more substrate, either tyramine HCl, p-cresol, or tyrosine. It would thus suggest that in the fraction *B* tyrosinase is normally inhibited or non-activated even though its amount may be increasingly great. Activation of *B* can also be accomplished by the addition of *A*, the 'naturally occurring activator(?)'. The growth of the enzyme during ontogeny is indicated in the figure and agrees essentially with that previously pointed out.[5] It is of some interest to again recall that the maximum amount of the enzyme is produced early in ontogeny, *i. e.*, during the first 21 days, and stays relatively constant thereafter. The question of naturally occurring substrate is of interest since these embryos apparently are not pigmented until after or upon hatching. From an inspection of the middle curve (substrate curve) in the figure it will be seen that substrate occurs naturally only late in embryonic development. It would thus seem that the embryo's content of tyrosinase is produced long before the substrate upon which it normally reacts is formed.

The question of the activation of the enzyme tyrosinase is of some further interest in view of the fact that in many cases its presence in tissues has been often questioned. It would seem from the present results that the enzyme may be present, but lacking an activator or substrate, no indications of its activity are evident.

9604 P

Deposition of Amyloid in Kidneys with Restricted Circulation.

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In an earlier report,[1] one of us described a method for production of renal insufficiency in rabbits, by reducing the blood flow through the renal artery of one of the kidneys by means of a loop of thread of a definite diameter. The loop is put on when the animals are small, the artery grows up to the size of the loop, and thereafter growth of the kidney stops. The other kidney continues to grow, and it is removed at some time later with the resultant production of renal insufficiency.

It has been our experience that the rabbits will survive if the restricted kidney represents 30% or more of the total previous kidney mass; in other words, if the restricted kidney is about half or more of the mass of the one removed. A group of 6 such animals survived for periods varying from 1½ to 10 months. The remaining kidney in each of these animals was swollen and pale, and microscopic examination revealed the presence of amyloid in the glomerular tufts and about the intertubular capillaries. This infiltrating substance was identified as amyloid by the methyl violet, congo red, and iodine staining methods. No amyloid was found in other organs.

No amyloid was found in the kidneys of a group of 10 rabbits surviving, one month or less, the removal of the normal kidney. Furthermore, no amyloid was found in the kidneys with restricted blood supply where the normal kidney was not removed.

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[1] Drury, D. R., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 856.

9605 P

Experimental Street-Virus Rabies in White Mice. Studies on Passive Immunization. I.*

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The problem of passive immunization against rabies has aroused relatively little interest in recent years. Most of the previous work on this subject is summarized by Kraus, Gerlach and Schweinburg.[1] Our department[2] has published results of experiments on passive protection against fixed-virus infections. Four consecutive passive-protection tests against street-virus rabies in white mice are herewith reported.

The source of the virus† employed was a fresh specimen of dog's brain, which contained Negri bodies. This was minced and preserved by refrigeration in 50% glycerol. Further preparation consisted of grinding to a 1/10 dilution in saline and centrifuging lightly for a few minutes. The supernatant fluid was inoculated intracerebrally into white mice in doses approximating 0.02 cc.

Antirabic serums were prepared as previously reported,[2] by immunizing goats and rabbits with fixed virus. The resultant antiserums, which were filtered and inactivated before use, contained neutralizing antibodies against street-virus. Antiserum was injected intraäbdominally in 2 doses of 1 cc. each, 2 days and one day before the intracerebral inoculation of virus. Untreated controls or mice that received corresponding amounts of normal serum were included in all experiments. The passive-protection tests included a total of 60 treated and 70 control mice. Observations were made at intervals of 12 hours during the critical periods of these experiments.

All controls developed symptoms of rabies with surprising regularity and died, the mean incubationary period for this group being 10.6 days. Impression-preparations from the brains of some of these mice showed Negri bodies. Forty-three percent of the treated mice were well at the end of 3 weeks and were classified as survivors.

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* This work was made possible through a grant from Mrs. John Sherman Hoyt.

[1] Kraus, R., Gerlach, F., and Schweinburg, F., *Lyssa bei Mensch und Tier*, Urban & Schwarzenberg, Berlin, 1926, p. 276.

† Virus was obtained through the courtesy of C. W. Arthur of the Pasadena City Health Department.

[2] Hoyt, A., Fisk, R. T., Moore, F. J., and Tracy, R. L., *J. Inf. Dis.*, 1936, **59**, 152.

These survivors comprised the total treated group of one test, whereas the 27 controls in this series sickened with a mean incubationary period of 11.0 days. Antirabic rabbit-serum was utilized in this experiment alone, goat's serum being employed in the other instances. All mice which were treated with goat's serum died but their mean incubationary period was lengthened to 14.5 days.

9606

Effects of Splenectomy on Pituitary Gonadotropic Substances.

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The recent reports of Gordon, *et al.*,[1] that splenectomized young rats are far more responsive than normal rats to continued treatment with the gonadotropic substance from pregnant urine, leads one to wonder if this is a specific reaction for such extracts or whether the same results could be obtained with pituitary glands.

Young female albino rats 25 days of age were given one male rat pituitary gland as a graft in the leg muscles or intraperitoneally each 5 days for a period of 25 days (either method of grafting gives the same result).[2] At 50 days of age they (Group A, Table I) were castrated and the ovarian weights compared to those from rats with identical treatment except that total splenectomy was performed at 25 days of age (Group B, Table I). These weights averaged 199 and 207 mg. respectively. The range was large in both as shown in Table I and this also is apparent in the large coefficient of variation. Therefore the mean difference divided by the standard error of the difference is not significant, the figure being only 0.2 (Table I) instead of 3 or more.

In a third group (Group C) the recipient rats were splenectomized, grafted with one male rat pituitary gland and castrated 5 days later. The ovaries weighed 18.4 which is similar to the 17.4 mg. obtained in Group D which were grafted but not splenectomized. Thus splenectomy had no apparent effect on the response of the ovaries to pituitary implants of 5 days duration.

In the last group the donors were splenectomized 3 weeks before

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[1] Gordon, Albert S., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 484; *Science*, 1937, **86**, 62.

[2] Emery, Frederick E., *Am. J. Physiol.*, 1937, **118**, 316.

TABLE I.

Ovarian weights in milligrams. All groups started at 25 days of age.
Groups A and B castrated 25 days and all other groups 5 days after the first implant.

Groups	Types of Donors	Recipients and Weights of Ovaries						
		No. of Rats	Splenectomized	Range	Mean	Median	$\frac{\text{Mean Diff.}}{\text{S.E. of Diff.}}$	Coef. of variation
A	Normal ♂	16	no	26 to 479	199.1	142	0.2	67
B	,, ,,	17	yes	46 to 489	207.4	206		61
C	,, ,,	12	yes	9 to 42	18.4	21		48
D	,, ,,	25	no	9 to 52	17.4	15	0.2	58
E	Splenectomized	12	no	9 to 40	18.0	13		42

they were sacrificed for the pituitary glands. The response of the ovaries of the recipients to one male rat pituitary was again not unlike the normal, the figures for the mean ovarian weight being 18.0 and 17.4 mg., respectively, in the two groups (Groups E and D, Table I). The range of ovarian weights is likewise similar in the two groups as is also the coefficient of variation. If emaciation occurs the testes may degenerate and cause a more potent pituitary but in these rats such extreme symptoms were not observed.

Gordon, *et al.*, explained their results as due to lack of anti-hormone formation in the splenectomized recipients. This allowed larger ovarian growth to occur. After a young rat has been stimulated to sexual maturity with pituitary grafts there supervenes an inhibition to further positive vaginal smears for a variable time depending upon the potency of the original grafts. A second graft during this period of inhibition produces ovarian growth but frequently no cornification of the vagina.[3] Whatever mechanism involved may be it was not altered by splenectomy in the groups shown in Table I. Each rat was subjected to 4 grafts after the first one at 5, 10, 15, and 20 days respectively. In Group A the total number of such occurrences was 64, of which 35 gave positive vaginal smears and 29, or 45%, were negative. In the splenectomized rats (Group B) the total number of such tests was 68, of which 23 were positive and 45, or 66%, were negative. The splenectomized rats were in this experiment equally or even more refractory than the normal recipients in vaginal reaction following each pituitary graft.

Summary. Tests with pituitary grafts of minimal strength failed to show that splenectomy was concerned with: (1) summation effects of ovarian weights produced by repeated grafts over a period of 25 days, (2) the amount of ovarian response obtained at the end of 5 days, (3) the potency of the pituitary gland of the donor, or (4) the inhibition of estrus following sexual maturity induced by pituitary grafts.

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[3] Emery, Frederick E., *Endocrinology*, 1933, **17**, 64.

9607

Determination of Minute Amounts of Type I Pneumococcal Polysaccharide by the Complement-Fixation Method.

KWAN-HUA LEE AND BACON F. CHOW.

From the Department of Biochemistry, Peiping Union Medical College, Peiping, China.

A quantitative method for the determination of a precipitinogen is valuable in the study of the mechanism of the precipitative reaction.[1] Heidelberger[2] determined the concentration of a solution of the polysaccharide of Type III pneumococcus by analyzing the amount of antibody-nitrogen precipitable from a previously standardized homologous serum. Obviously, such a method requires considerable material and is not applicable to minute amounts of polysaccharide. In the course of our study of the mechanism of the precipitin-reaction of Type I pneumococcus it was necessary to devise a method for the determination of a very small amount of polysaccharide. Since the polysaccharide united with its homologous rabbit-precipitin can fix complement,[3] it seemed advisable to investigate the possibility of adapting this method to our needs. We report the details of this method which permits the estimation of as little as 0.00001 mg. of Type I pneumococcal polysaccharide even in the presence of homologous antibody.

The usual procedure of complement-fixation was followed: 0.5 cc. of Type I antipneumococcal rabbit serum plus 0.5 cc. of the homologous polysaccharide in various dilutions was incubated at 37° for 30 minutes. After the addition of 1.0 cc. of guinea pig's complement containing 2 units per cc. it was placed in an icebox overnight. The following morning, the tubes were warmed to room temperature and 0.5 cc. of hemolysin (2 units) and 0.5 cc. of 2% sheep-cell suspension were added. The mixture was incubated at 37°C. for one hour. In order to estimate the concentration of an unknown solution, a solution of known concentration of polysaccharide was used for comparison.

This method is particularly useful in the following 3 cases:

Case I. When there is no interfering substance. When the unknown contains only a minute amount of polysaccharide it can be easily estimated by comparing the titer of the unknown with that

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[1] Hooker, S. B., and Boyd, W. C., *J. Gen. Physiol.*, 1935, **19**, 373.

[2] Heidelberger, M., *J. Exp. Med.*, 1932, **55**, 555.

[3] Goodner, K., and Horsfall, F. L., *J. Exp. Med.*, 1936, **64**, 201.

TABLE I.

Effect of Rabbit-precipitin on the Complement-fixation Titer.

Exp.	0.25 cc. IRS (1 + 2) +	Final dilution of polysaccharide 1:1,000,000	1:2,000,000	1:4,000,000	1:8,000,000	1:16,000,000	1:32,000,000
I	0.25 cc. saline	++++	++++	++++	++++	—	—
II	0.25 cc. RP (2 mg. protein/cc.)	++++	++++	++++	++++	—	—
III	0.25 cc. RP (0.5 mg. protein/cc.)	++++	++++	++++	++++	—	—

The components used in each experiment are given below:

Exp. I 0.5 cc. of polysaccharide + 0.25 cc. IRS + 0.25 cc. 0.85% NaCl.
II 0.5 " " " + 0.25 " IRS + 0.25 " of RP (2 mg. protein/cc.)
III 0.5 " " " + 0.25 " IRS + 0.25 " of RP (0.5 mg. protein/cc.)

Where IRS = immune rabbit serum.
RP = **rabbit-precipitin.**

TABLE II.

Effect of Horse-precipitin on the Complement-fixation Titer.

Exp.	0.25 cc. IRS (1 + 4) +	Final dilution of polysaccharide 1:1,600,000	1:32,000,000	1:64,000,000	1:128,000,000	1:256,000,000
I	0.25 cc. of 0.85% NaCl	++++	++++	++++	—	—
II	0.25 cc. HP (2 mg. protein/cc.)	++++	++++	++++	—	—
III	0.25 cc. HP 0.25 mg. protein/cc.)	++++	++++	++++	—	—
IV	0.25 cc. IRS (1:25) 0.25 cc. 0.85% NaCl	++++	++++	++++	++++	—
V	0.25 cc. HP (0.25 mg. protein/cc.)	++++	+++	—	—	—

The components used in the experiments are given below:

	cc. of polysaccharide solution		cc. of IRS		
I	0.5	+	0.25	(1:5)	+ 0.25 cc. 0.85% NaCl.
II	0.5	+	0.25	(1:5)	+ 0.25 cc. HP 0.25 mg. protein/cc.
III	0.5	+	0.25	(1:5)	+ 0.25 cc. HP 0.06 mg. " "
IV	0.5	+	0.25	(1:25)	+ 0.25 cc. 0.85% NaCl.
V	0.5	+	0.25	(1:25)	+ 0.25 cc. HP 0.25 mg. protein/cc.

of a standard solution of polysaccharide. Examples of this kind are found, when it is necessary to find the rate of diffusion of the polysaccharide through a membrane.

Case II. When homologous rabbit-precipitin is also present. In studying the mechanism of the precipitin-reaction, it is often necessary to estimate the concentration of the polysaccharide in the presence of an excess of rabbit's precipitin. An experiment was performed to ascertain whether this method can be used in such a case.

The results (Table I) show that the presence of immune rabbit's precipitin, when not in great excess, may not alter the complement-fixation titer. Traces of the polysaccharide in the isolated precipitin used in this experiment were removed by precipitation of the antibody at half saturation of ammonium sulfate and dialysis against saline solution.

Case III. When horse-precipitin is present. Horse-precipitin inhibits the complement-fixation reaction and tends to give low results. The inhibitory effect can be overcome by using a higher concentration of rabbit's serum.

Table II shows that when the immune rabbit's serum was diluted only 5-fold (I, II, III) the inhibitory effect of the horse-precipitin was not observable. On the other hand, when the same serum was diluted 25-fold, the effect of the same amount of horse-precipitin was decidedly noticeable.

9608

Studies on the Mechanism of the Precipitin-Reaction. I. Behavior of Immune Precipitate Towards Washing.

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Several differences in the immunological activities of Type I antipneumococcal rabbit and horse sera have been reported.[1] However, little is known about the difference, if any, in the manner of union between the polysaccharide and its precipitin in the sera of these two animals. In this report, we shall show that such a difference exists. When the immune precipitates were washed with normal saline solution, and the amounts of polysaccharide present in the combined washings were estimated by the method described in the preceding paper, it was found that practically all the polysaccharide in the immune-rabbit precipitate could be accounted for in the washings. On the other hand, no appreciable amount of polysaccharide could be removed from the immune-horse precipitate.

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[1] Goodner, K., and Horsfall, F. L., *J. Exp. Med.*, 1936, **62**, 485.

Twenty cc. of Type I pneumococcal polysaccharide (0.1 mg. per cc.) were added to 20 cc. of homologous horse or rabbit antiserum. The mixture was incubated at 37°C. for half an hour. The supernate was poured off and was found to contain an excess of antibody. The precipitate was washed with four 50-cc. portions of 0.85% NaCl solution, dissolved in 5 cc. of water and 5 cc. of N/140 NaOH, and the alkaline solution was poured into 400 cc. of 0.85% NaCl solution (designated as P). The polysaccharide-content of the P-fraction and the combined washings (W) was determined by the complement-fixation method. The results are shown in Table I.

TABLE I.
Polysaccharide Contents of the Washed Immune Precipitate and Washings.

Animal origin		Total polysaccharide found mg.	Total protein mg.
Horse	P	2.0	40
	W	none	20
Rabbit	P	0.05	50
	W	1.8	25

In another experiment, the washed immune-rabbit precipitate containing 50 mg. protein was found to contain 0.025 mg. polysaccharide. The precipitate was suspended in 20 cc. of water and dissolved with 5 cc. of N/70 NaOH. The clear alkaline solution was brought to slight turbidity with N/70 HCl and allowed to stand in ice-water overnight. On the following morning the solution was neutralized, solid NaCl was added to 0.85%, and it was centrifuged. The supernatant fluid which contained 25% of the original protein was found to have a higher titer in agglutination, precipitation with homologous polysaccharide, and mouse-protection, than the combined washings. We have found that the addition of 0.025 mg. polysaccharide to 50 mg. of immunologically pure precipitin[2] caused no visible precipitation.

We have stated elsewhere[2] that the low immunological activity of the antibody-polysaccharide complex is due to some alteration in the antibody-protein itself and not merely to the combination of the antibody with the polysaccharide. If the antibody had not been altered during precipitation, the protein in the washings of immune-horse precipitate which contained no polysaccharide should have the same activity as pure precipitin. But this is not the case. Furthermore, it is difficult to conceive that the immune-rabbit precipitate after losing nearly all its polysaccharide should still retain the original low solubility and low activity of the precipitate. The increase in solubility and immunological activities of the washed precipitate can be brought about not by the removal of the polysaccharide but by treating with alkali under conditions suitable for the reversion of denatured protein.

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[2] Chow, B. F., and Wu, H., *Chinese J. Physiol.*, 1937, **11**, 139.

9609

Fractionation of Antibody in Type I Antipneumococcal Serum by Addition of Ammonium Sulfate.

Kwan-hua Lee, Bacon F. Chow and Hsien Wu.

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It has been reported[1] that the antibody of Type I pneumococcus in horse or rabbit serum is separable into fractions with different immunological and physical properties and that this separation can be effected by absorption with Type I pneumococcal polysaccharide. In this paper we shall present evidence that the antibody of Type I pneumococcus is separable into fractions by precipitation with ammonium sulphate in such a manner that only a small portion of the antibody comes down in each successive precipitation.

The results of a typical experiment are given below:

To 100 cc. (12.0 mg. N/cc.) of Type I antipneumococcal rabbit serum were added 34 cc. of saturated ammonium sulfate solution. To hasten flocculation the suspension was warmed to about 37°C. It was centrifuged, and the supernate was poured into another tube to which another portion of saturated ammonium sulfate solution was added according to the scheme given in the accompanying diagram. The precipitates were dissolved in water, dialyzed against 0.005 M acetate buffer at pH 5.0, and centrifuged. Both the supernate and the precipitate were adjusted to the usual saline concentration, and the immunological activities were tested. The percentage of protein-nitrogen present in each fraction is given in Table I.

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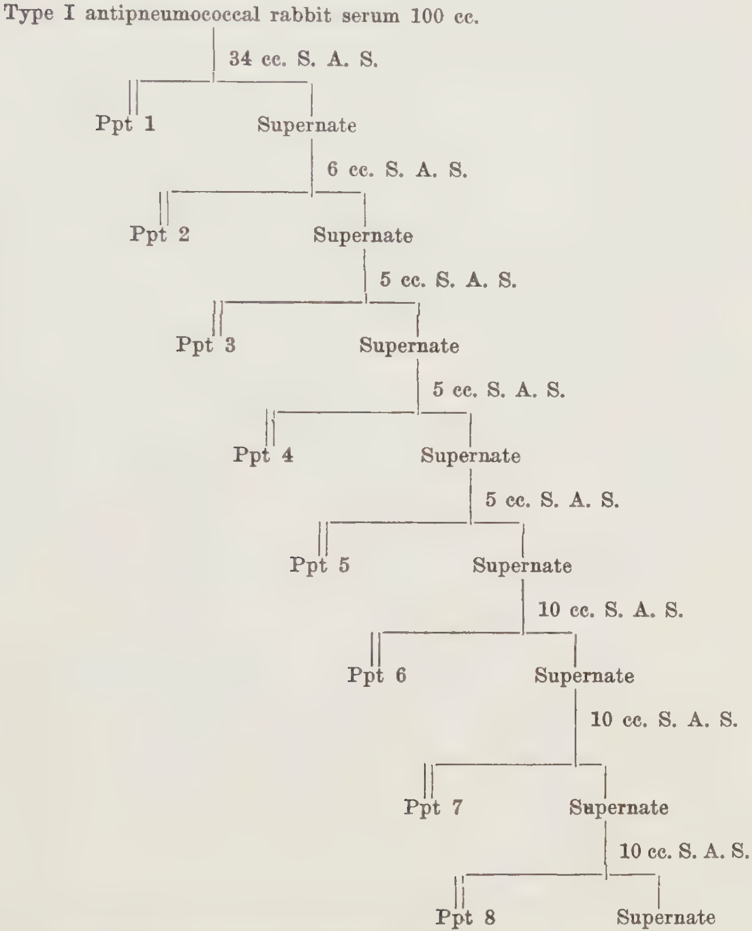
[1] Chow, B. F., *Proc. of the Chinese Physiol. Soc.*, 1937, 18.



S. A. S. denote saturated ammonium sulfate solution.
Ppt denote precipitates.

TABLE I.

No.	Insoluble at pH = 5.0		Soluble at pH = 5.0	
	Mg. N found	% of original	Mg. N found	% of original
1	1.06	0.88	74.8	6.16
2	0.33	0.28	238.4	19.80
3	0.17	0.14	105.0	8.75
4	0.56	0.47	57.6	4.75
5	0.81	0.67	23.6	1.96
6	0.90	0.75	19.8	1.65
7	0.41	0.33	23.6	1.96
8	0.48	0.40	23.4	1.95

TABLE II.

Agglutination

No.	Mg. N protecting mice from 1,000,000 MLD of Type I pneumococcus	A/2	A/8	A/16	A/32	A/64	A/128
1	0.011	++++	++++	++++	++++	++	+
2	0.012	++++	++++	++++	++++	++	—
3	0.006	++++	++++	++++	++++	+++	+
4	0.004	++++	++++	++++	++++	+	—
5	0.006	++++	++++	++++	+	—	—
6	0.006	++++	++++	++++	+	—	—
7	0.012	++++	++++	++	+	—	—
8	0.110	++++	++++	+	—	—	—

A = 0.4 mg. N/cc.

Precipitation

Final dilution of original polysaccharide

No.	Mg. N protecting mice from 1,000,000 MLD of Type I pneumococcus	1:4,000	1:16,000	1:64,000
1	0.011	++++	++++	++++
2	0.012	++++	++++	++++
3	0.006	++++	++++	++++
4	0.004	++++	++++	++++
5	0.006	++++	++++	++++
6	0.006	++++	++++	++
7	0.012	++++	+	—
8	0.110	—	—	—

Final dilution of polysaccharide* hydrolyzed by acid

No.	Mg. N protecting mice from 1,000,000 MLD of Type I pneumococcus	1:4,000	1:16,000	1:64,000
1	0.011	++++	++++	++++
2	0.012	++++	++++	++++
3	0.006	++++	+	—
4	0.004	++++	+	—
5	0.006	+++	+	—
6	0.006	—	+	—
7	0.012	—	—	—
8	0.110	—	—	—

Final dilution of polysaccharide* hydrolyzed by both acid and alkali

No.	Mg. N protecting mice from 1,000,000 MLD of Type I pneumococcus	1:4,000	1:16,000	1:64,000
1	0.011	++++	++++	++++
2	0.012	++++	++++	+
3	0.006	+++	+	—
4	0.004	—	++	—
5	0.006	—	—	—
6	0.006	—	—	—
7	0.012	—	—	—
8	0.110			

*The hydrolysis of the original polysaccharide was carried out in the same manner previously[6] described.

The insoluble portion represents only a very small fraction of the serum-globulin, and was only partially soluble in saline solution. Therefore its immunological activities were not studied.

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[6] Chow, B. F., *J. Exp. Med.*, 1936, **64**, 843.

Although all these 8 fractions soluble at pH 5.0 could agglutinate Type I pneumococci, they differed not only quantitatively but qualitatively in their reactivity with the homologous polysaccharide and its hydrolytic products. The results of qualitative agglutination and precipitation of these fractions (Table II) reveal the following facts: First, all 8 fractions contain antibodies, for they agglutinate Type I pneumococci and protect mice from an otherwise lethal dose of the organism. Secondly, fraction 8 corresponds to the antibody[2] obtained by the agglutination of Type I antipneumococcal rabbit serum previously absorbed with the homologous polysaccharide. It possesses both agglutinative and protective power but fails to react with the homologous polysaccharide. Thirdly, hydrolysis of the original polysaccharide by acid or both acid and alkali destroys the reactivity with certain fractions of the antibody. These fractions (*e. g.,* Nos. 5 and 6) obtained by the addition of ammonium sulfate gave a heavy precipitate with the original polysaccharide, but only a slight precipitate with the product hydrolyzed by acid and practically none with the product hydrolyzed by both acid and alkali.

The extensive study on the fractionation of normal serum-globulin with salts by Sørenson[3] and other workers supports the current view that it is an easily dissociable complex consisting of the so-called euglobulin and pseudoglobulin in varying proportions. Numerous studies on the distribution of antibodies in antipneumococcal sera have been made by fractionation with ammonium sulfate. However, the estimation of antibody in each fraction is usually made by mouse-protective tests[4] or by the precipitative reaction[5] on the assumption that the same antibody is present in each fraction. This has been found not to be the case in the present study.

9610 P

A 2,4-Dinitrophenylhydrazine Derivative of Dehydroascorbic Acid and the Estimation of Vitamin C.

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When dehydroascorbic acid is treated with a saturated solution of 2,4-dinitrophenylhydrazine in N HCl, a reddish crystalline derivative is formed. The reaction is catalyzed by acetic acid. Analysis of the product obtained showed it contains two 2,4-dinitrophenylhydrazine groups in its molecule. The derivative is apparently an osazone of dehydroascorbic acid with the hydrazine groups attached to carbon atoms 2 and 3. The compound melted at 257-259° (corrected) and microanalysis showed it has the following composition: $C_{18}H_{14}O_{12}N_8$

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[2] Sørensen, S. P. L., *Compt. rend. trav. lab.*, Carlsberg, 1923-25, **15**, No. 11.

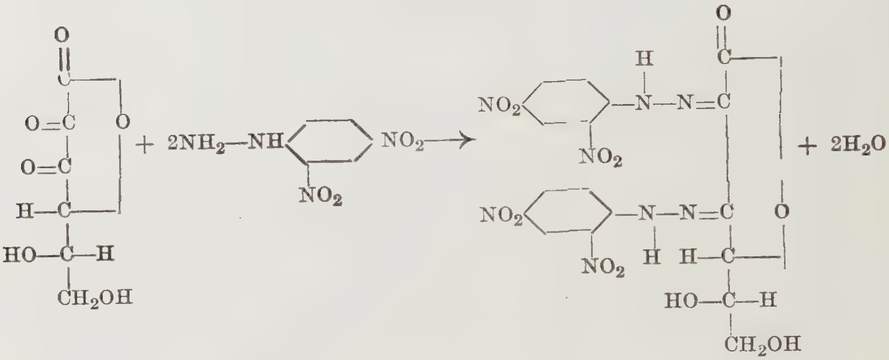
[3] Chow, B. F., and Wu, H., *Chinese J. Physiol.*, 1937, **11**, 163.

[4] Felton, L. F., and Kaufmann, G. J., *Immunology*, 1933, **25**, 165.

[5] Chow, B. F., PROC. SOC. EXP. BIOL. AND MED., 1937, **34**, 651.

Calculated: C 40.43, H 2.64, N 20.98.
Found: " 40.40, " 2.88, " 21.11.

The evidence indicates the reaction is as follows:



This compound was prepared as follows: 0.2 gm. of pure ascorbic acid in 200 cc. of 10% acetic acid was shaken with norit and filtered. Two volumes of 2,4-dinitrophenylhydrazine in N HCl were added. The mixture was allowed to stand at room temperature for 72 hours and then centrifuged. The precipitate was washed 6 times with distilled water.

Advantage was taken of this reaction to make it the basis of a specific method for the determination of vitamin C. The dehydroascorbic acid osazone is reduced to a colorless compound by warming with 12% HCl containing 10% $SnCl_2$ and the compound is hydrolyzed by heating with these reagents in an autoclave at 15 lb. pressure. Under the latter conditions the released dehydroascorbic acid is converted into furfural, which, upon cooling, is determined by the colorimetric aniline acetate method[1] previously published. Use is made of a standard solution of pure ascorbic acid which is treated in the same way as the acid extract of tissue.

Substances most likely to interfere in this method are free pentoses which will give a centrifugable precipitate in concentrations greater than one mg. per cc. As the vitamin is extracted from tissues by a procedure which usually involves a 10-fold dilution, and greater dilutions may be made, this possible difficulty with tissues containing free pentose is readily overcome. The method appears to be entirely specific for the estimation of vitamin C.

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[1] Roe, J. H., *J. Biol. Chem.*, 1936, **116**, 609.

Osazone formation does not occur in the first few hours after mixing 2,4-dinitrophenylhydrazine with ascorbic acid but later crystals appear slowly. This behavior is interpreted as indicating that ascorbic acid does not react with the hydrazine under these conditions, but upon standing it is oxidized to dehydroascorbic acid which reacts to produce the compound described.

This procedure is particularly advantageous for the determination of vitamin C in urine, for which the indophenol and other oxidation-reduction methods lack specificity. Ascorbic acid added to urine is recovered quantitatively by this method.

In preparing this paper the report of Stewart, Scarborough and Drumm[2] came to our attention. These authors have made a 2,4-dinitrophenylhydrazine derivative from the ascorbic acid in urine. As the method of preparation was not stated and no analyses were reported, a comparison of their derivative with ours cannot be made. The melting point reported (269-271 uncorrected) is about 20 degrees higher than that of our product.

The details of our procedure for the estimation of vitamin C will be published later.

9611

Glycerol Toxicity and Hemoglobinuria in Relation to Vitamin C.

Carl Pfeiffer and I. Arnove. (Introduced by E. M. K. Geiling.)
From the Department of Pharmacology, University of Chicago.

In view of the several recent reports[1, 2, 3] of the curing of paroxysmal hemoglobinuria by the administration of ascorbic acid, it was thought that a study of ascorbic acid in relation to experimental hemoglobinuria might be of interest. Of the numerous substances which produce hemoglobinuria it was found that parenterally injected glycerol was the most practical.

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[2] Stewart, C. P., Scarborough, H., and Drumm, P. J., *Nature*, 1937, **140**, 282.

[1] Armentano, L., *Nature*, 1936, **137**, 910.

[2] Lotze, H., *Klin. Woch.*, 1936, **15**, 941.

[3] Armentano, L., and Beutsath, A., *Klin. Woch.*, 1936, **15**, 1594.

When glycerol was administered subcutaneously to 150 gm. white rats of a homogeneous strain, it was found (Table I) that the smallest dose producing hemoglobinuria, as shown by spectrographic analysis of the urine, was 0.75 cc. per kg. where 4 out of 12 rats had hemoglobinuria, while 14 out of 22 rats (64%) had hemoglobinuria at 1.0 cc. per kg. When the glycerol was antidoted by a subsequent dose of ascorbic acid, 72% had hemoglobinuria at 1.5 cc. per kg. of glycerol. Because the glycerol is very rapid in producing hemoglobinuria it was thought that the prophylactic administration of the ascorbic acid might afford greater protection against the hemoglobinuria. Accordingly, when the ascorbic acid was given first, it was found that only 8 out of 12 (66%) had hemoglobinuria when a larger dose of glycerol (2 cc.) was injected.

TABLE I.

Drug	Dose	Dose	Result	Hemoglobinuria	Color of Urine
Glycerol*	0.75 cc./Kg.		4/12†	Trace	Pink
"	1.00 " "		14/22	++	Red
"	7.00 " "		8/10 Lived	++++	"
"	8.00 " "		5/20 "	++++	"
" I.P.	4.00 " "		9/10 "	++++	"
" I.P.	5.00 " "		6/10 "	++++	"
Glycerol and ascorbic acid	1.25 " "	500 mg./Kg.	4/16	Trace	Green
Glycerol and ascorbic acid	1.50 " "	500 " "	26/36	"	"
Ascorbic acid and glycerol	500 mg./Kg.	1.5 cc./Kg.	2/6	"	"
Ascorbic acid and glycerol	500 " "	2.0 " "	8/12	"	"
Glycerol and ascorbic acid	8.00 cc./Kg.	300 mg./Kg.	2/4 Lived	++++	Brown
Glycerol and ascorbic acid	8.00 " "	500 " "	6/7 "	++++	"
Glycerol and ascorbic acid	8.00 " "	600 " "	3/4 "	++++	"

* All doses were administered subcutaneously unless otherwise designated.

† Should be read 4 out of 12 rats had hemoglobinuria. All rats had 10 cc. of 0.9% NaCl solution intraperitoneally for diuretic purposes. The glycerol was diluted to 10-50% with 0.9% NaCl solution before administration. All ascorbic acid was neutralized with 50% of its weight of $NaHCO_3$ immediately before administration.

That the ascorbic acid is useful in decreasing the percentage mortality at the minimal lethal dose (8 cc. per kg.) is shown by the reduction in mortality from 75% (5/20 lived) to 27% (11/15 lived) when 300 to 600 mg. per kg. ascorbic acid was used). The strong reducing action of the ascorbic acid is possibly responsible for the partial conversion of the oxy- and met-hemoglobin in the urine to green degradation products, but this does not appreciably alter the typical spectrographic lines. Traces of hemoglobin in the presence of these green reduction compounds are easily overlooked unless spectrographic analysis is used.

The mode of action of this antagonism has not been determined. This study, however, adds to the list of toxins and toxic substances which ascorbic acid aids in detoxifying.[4-7] It is of importance to note that many of the biological compounds reported as antagonistic to vitamin C contain as much as 50% of glycerol.[8, 5, 7] Since glycerol compounds most probably occur in the animal body in the phagocytosis of tubercle bacilli,[9] this antagonism may be of importance in accounting for the increased ascorbic requirement of tuberculous patients.[10] The use of glycerol to produce more extensive tuberculosis in white rats[11] is also of interest in view of the above data.

Summary. The administration of ascorbic acid prior to a parenteral dose of glycerol will raise the dose necessary to produce hemoglobinuria by 100% or more. The ascorbic acid may be effective in lowering the mortality rate from toxic doses of parenteral glycerol.

The authors wish to express their appreciation for the helpful suggestions of Dr. E. M. K. Geiling.

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9612

Cutaneous Reactivity to Tuberculin in the Dog.*

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The prevailing opinion expressed in the current literature[1-4] is that tuberculous dogs react irregularly or not at all to injections of tuberculin. This opinion is apparently based primarily upon the reports of Cobbett and Griffith,[5] who estimated the sensitivity of tuberculous dogs by determining the thermal changes following the injection of 2 to 5 cc. of tuberculin. The dogs were infected with human, bovine or avian types of tubercle bacilli injected intravenously, intraäbdominally or subcutaneously in doses ranging from 1 to 50 mg. A few of the dogs were infected by feeding.

During the course of studies of experimental pulmonary tuberculosis in dogs we have had the opportunity to observe the cutaneous reactivity to tuberculin from the time of initiation of the lesion throughout the various stages of healing or progression of the disease. Our findings with regard to the reactivity of the skin of tuberculous dogs after intracutaneous injection of tuberculin have been contrary to prevailing opinion. These reactions appear to be more constant than those obtained after subcutaneous injection.

Normal dogs were infected by the intrapulmonary injection of varying doses of virulent human or bovine type† tubercle bacilli sus-

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* Aided by a grant from the Medical Research Council of the American Medical Association.

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† The strains of human and bovine type employed were isolated in 1930, the human strain from a fatal case of tuberculous meningitis and the bovine from a fatal case of glandular and osseous tuberculosis. The human strain has been shown to possess a high degree of virulence for guinea pigs and the bovine strain for both guinea pigs and rabbits. The organisms are carried on glycerin-egg-yolk medium.

pended in mucin. The injections were made through a ureteral catheter inserted deep in a lower-lobe bronchus by means of a bronchoscope. The dosage of bacilli varied from 0.075 mg. to 0.5 mg. per kilo or total doses of 1 to 5 mg. per dog. The estimation of the quantity of bacilli used for injections was made by centrifuging in a Hopkins tube. Mucin was used in preparing suspensions to insure a more compact inoculum and to reduce the tendency to widespread bronchial dissemination or expulsion of the material by coughing. Comparisons of the pathogenicity for guinea pigs of tubercle bacilli suspended in gastric mucin[6] with saline suspensions have, in the doses employed, demonstrated no significant differences in the course of the disease produced by the two suspensions.[7]

Cutaneous sensitivity was determined by measuring the reaction to 0.1 cc. amounts of varying dilutions of standard "Old Tuberculin" (O.T., Parke Davis) and Purified Protein Derivative (P.P.D., Seibert)‡ injected intracutaneously. The tests were made upon carefully shaved areas of the skin of the neck and upper portions of the hind quarters of the dog after thorough cleansing with soap and water and alcohol. The injections were made with 27 gauge needles and the raised blebs were ringed with a skin-pencil. This greatly facilitated reading the tests especially in pigmented skin or when weak or negative reactions were encountered.

Dilutions of both O.T. and P.P.D. were made with 0.85% saline and in all cases were kept in the refrigerator and employed within 3 days after diluting. One-tenth cc. of a 1:100 dilution was chosen as the standard test-dose. Other dilutions varying from 1:10 to 1:100,000 were also employed when indicated by the reactions to the standard test. Readings of the tests were made at 24, 48, and 72 hours and the 48-hour readings were graded according to the suggestion of Aronson:[8]

Slight redness without edema	—
Slight redness with edema measuring less than 5 mm.	?
Edema measuring 5 to 10 mm.	+
” ” 10 to 20 ”	++
” ” 20 to 30 ”	+++
Central necrosis	++++

Results. A series of 30 normal dogs tested before experimental infection were uniformly negative in their reactions to tuberculin; in no case have reactions in any way similar to the typical tuberculin-reaction been encountered. Forty-two dogs with various types of experimental infection, including 10 dogs inoculated with B.C.G., have given positive reactions with 1:100 tuberculin. This figure represents 93.6% of infected and vaccinated dogs studied. The majority of these dogs became positive early and were definitely and consistently positive (2 to 3 plus) with 1:100 tuberculin. Dogs vaccinated with B.C.G. (2.5 to 10 mg.) became skin-reactive after a longer interval than in the case of the virulent strain and often gave weak or irregular reactions. Weak, irregular or negative reactions when encountered in dogs infected with virulent bacilli have in general been associated with rapidly progressing or far-advanced tuberculosis. Six dogs previously positive to tuberculin became weak or negative before death; necropsies revealed extensive tuberculosis. Three, or 6.4%, of infected or vaccinated dogs studied gave doubtful or negative reactions to tuberculin. One of these, necropsied 98 days after infection, showed no gross evidence of tuberculosis; the other 2 (one injected intravenously with 5 mg. B.C.G., the other with 1 mg. of human tubercle bacilli into the right lower lobe) have not been necropsied.

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‡ The Purified Protein Derivative employed in these experiments was procured from Dr. Florence Seibert through the courtesy of Dr. E. R. Long.

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TABLE I.
Skin Reactivity to Tuberculin in the Dog.

No. tested	Infection and vaccination data			Skin sensitivity 1:100 O.T. and P.P.D.
	Type of bacilli	Dose (mg.)	Route	
12	Human	1.0	Intrapulmonary	1 to 3 plus
3	”	3.0	”	2 ” 3 ”
5	”	4.0	”	1 ” 3 ”
8	”	5.0	”	2 ” 3 ”
2	Bovine	2.0	”	1 ” 3 ”
2	”	5.0	”	2 ”
1	B. C. G.	2.5	”	1 ” 2 ”
2	”	4.0	”	1 ” 2 ”
2	”	4.0	Subcutaneous	1 ” 2 ”
5	”	5.0 to 10.0	Intravenous	1 ” 2 ”
2	Human	1.0	Intrapulmonary	Negative*
1	B. C. G.	5.0	Intravenous	” (47 days)
30	Normal controls	0	0	Negative

Summary:

Total No. infected dogs tested	45
” ” ” ” with positive reactions	42, or 93.6%
” ” normal control dogs tested (negative reactions)	30

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* One of these dogs showed no gross evidence of tuberculosis at autopsy.

Fourteen of the 16 tuberculous dogs which manifested strong (2 or 3 plus) reactions to 1:100 tuberculin were also tuberculin-positive when tested with 1:1000 dilutions. Four of 6 dogs tested have reacted positively to dilutions of 1:10,000. No reactions have been elicited in the few cases in which dilutions of 1:100,000 tuberculin have been employed. No reactions showing central necrosis (4 plus) have been seen. In general comparable dilutions of O.T. and P.P.D. as employed in these experiments have elicited similar reactions. Further data concerning the comparison of the two types of tuberculin will be published at a later date.

Summary. Thirty normal dogs tested repeatedly with 1:100 tuberculin have given negative reactions. Forty-two dogs with tuberculous lesions have given positive reactions to the intracutaneous injection of tuberculin in doses of 1:100 to 1:10,000. Three dogs gave doubtful or negative reactions; one of these animals has been necropsied and showed no gross evidence of tuberculosis. Six dogs previously positive to tuberculin have given negative or doubtful reactions from 1 to 3 months before death; necropsies invariably revealed extensive tuberculosis. Other than this there has been no apparent correlation between the degree of cutaneous sensitivity and the extent of the tuberculous lesion.

9613

Observations on *Bacterium melaninogenicum:* Demonstration of Fibrinolysin, Pathogenicity and Serological Types.*

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A review of the literature on *Bact. melaninogenicum*[1-4] reveals an uncertainty as to its pathogenicity and as to the existence of more than one serological type. Evidence is here presented that the proteins extracted from 2 strains of this organism are different in their chemical and antigenic properties and that pathogenicity can be demonstrated under certain conditions.

Two strains were studied: The first (py) was isolated from the gums of a patient suffering from pyorrhœa and the second (M19), from the lung of a monkey which had developed a pulmonary abscess after intrabronchial inoculation with infectious material.[5] Both cultures conformed to the description given by Shevky, Kohl and Marshall[3] and Burdon.[1, 2] The first strain differed, however, from the second in its failure to liquefy gelatin and to form indol in peptone-water.

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*Presented before the American Association for Experimental Pathology, Memphis, Tennessee, April 22, 1937.

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The pathogenicity of pure cultures was tested by injecting dogs intrabronchially, white mice subcutaneously and rabbits intracutaneously.

With the aid of a fluoroscope, 2 dogs were injected intrabronchially with large doses of *Bact. melaninogenicum* suspended in a menstruum of starch.[6] One animal developed a febrile reaction. A rœntgenogram of the lung showed an area of increased density of the infected lobe.† The other had increased bronchial markings which lasted for at least a month.

Mice were inoculated subcutaneously in the groin with 0.05 to 0.1 cc. of broth culture of *Bact. melaninogenicum.* Edema and inflammation developed which persisted for 24 hours. Control animals, injected with heat-killed cultures, showed no demonstrable lesions.

Rabbits were injected intracutaneously with 0.02 cc. of staphylococcal toxin combined with 1/5 unit of Squibbs antitoxin; 48 hours later these sites were inoculated with living or heat-killed cultures of *Bact. melaninogenicum.* Inflammation, exudation and necrosis were observed only in areas infected with living cultures.

Experiments were conducted by the method of Tillett and Garner,[7] employing alcohol for precipitation and concentration and human fibrinogen and thrombin for the demonstration of fibrinolysin. Strain (M19) gave complete lysis in a 1:4 dilution after 40 minutes' incubation, while strain (py) showed partial (2+) lysis only in original concentrations.‡ The presence of fibrinolysin is considered by many authors as evidence of invasiveness or pathogenicity.[8–11]

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TABLE I.

Biochemical Properties of Proteins Extracted from Two Strains of *Bacterium melaninogenicum.*

Microörganism	Source	% Total N	Ash %*	Total N (Corrected for ash)	Biuret†	Xantho-Proteic†	Hopkins, Cole†	Ninhydrin†	Millon†	Molisch†	Bial-tollens‡	Precipitin titers§
Bact. melaninogenicum (M 19)	Pulmonary abscess	9.83	4.91	10.35	++	+++	+++	+++	+++	+++	++	1:128,000
Bact. melaninogenicum (py)	Pyorrhœa	14.28	5.17	15.07	+++	+++	+++	+++	+++	+++	—	1:64,000

*Microchemical analyses were performed under the direction of Prof. Paul L. Kirk, by Mr. H. C. Johnson, Department of Biochemistry, University of California, Berkeley.

†Tests performed on 1:1000 dilution.

‡Test for pentose.

§Maximal final dilutions of protein giving strong precipitation when added to an equal volume of homologous antiprotein rabbit serum.

Bacterial proteins, corresponding to fraction D, were extracted from 2 strains of *Bact. melaninogenicum* by the method of Heidelberger and Kendall.[12] The medium[13] was hormone-broth containing 1% cysteine hydrochloride with pieces of autoclaved brain, tied up in a parchment bag, placed at the bottom of the flasks.

The biochemical characteristics of the proteins are given in Table I. Both were antigenic when injected intravenously in repeated doses into rabbits, and non-toxic, when inoculated intra-abdominally into white mice.

Precipitin-tests were performed according to the technic of Lancefield,[14] employing various dilutions of the bacterial proteins and a constant dose of antibacterial and antiprotein sera. Absorptive tests were done, whenever indicated, with concentrated bacterial suspensions as adsorbing agents, since preliminary tests revealed that the proteic solutions were unsatisfactory for this purpose.

It was observed that the proteins extracted from the 2 strains of *Bact. melaninogenicum* are immunologically distinct. Cross-reactions were seen in antibacterial sera for *Strep. hemolyticus,* Group A, but these heterologous precipitins were readily removed by absorption with homologous bacterial suspensions and were not found in sera obtained by immunization with protein-solutions or with suspensions of several other bacterial species: *Fusobacteria,* types I, II and III, *Strep. evolutus, Pneumococcus III* and *Staphylococcus aureus.*

Immune rabbit-sera produced by repeated intravenous injections of the bacterial proteins contain specific anticarbohydrate as well as antiprotein. Absorption with the protein removed both antibodies but absorption with carbohydrate removed only the anticarbohydrate.

These findings are in agreement with those of Heidelberger and Kendall[15] on *Streptococcus hemolyticus* and suggest the existence of a conjugated carbohydrate-protein analogous to the artificial pneumococcus III-polysaccharide-protein synthesized by Avery and Goebel.[16]

Valuable assistance was rendered by Dr. Alex Kaplan, Mrs. D. G. Mercado and Mrs. J. D. Taranik in the chemical and serological investigations. E. R. Squibb & Sons furnished the staphylococcus toxin and antitoxin.

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9614

Gonadokinetic Effects in Parabiotic Rats.

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It has been shown[1] that in parabiotic rats the testes of a hypophysectomized partner could be completely maintained in or restored to a normal condition by gonad-stimulating hormone emanating from the pituitary of a castrated twin. It was also reported that the pituitaries of cryptorchid and of normal partners were in this respect much less potent than those of castrates. The data which will be presented in this report for the most part confirm and extend the previous work.

As regards the response of the testes of a hypophysectomized parabiotic twin it has been our experience that the following factors are of greatest importance in the final analysis: (1) The type of partner; whether castrated (regardless of sex), cryptorchid, or normal. (2) The time necessary for adequate vascular anastomosis between the partners. (3) The time necessary for a physiological process to become manifest or to run to completion.

Experience has shown that the scrotum in the rat reacts to testicular hormone much as do the accessory sexual organs.[2, 3] From the results of our former and present studies in parabiotic rats we have found it possible to conclude that the scrotum in a hypophysectomized parabiotic rat is qualitatively more sensitive to testicular hormone than are the prostate and seminal vesicles. This has proved to be an important criterion in our work on parabiotic rats, as a pair does not have to be sacrificed in order to discover from the condition of the accessory sexual glands and the interstitial cells of the testes whether or not testicular hormone is being produced. Further, our previous observations, confirmed by later results, have shown that within certain limits the size of the testes in a hypophysectomized parabiotic rat is an index to the degree of their gametogenic activity. This is especially true when the testes are large, since the interstitial tissue at best forms but a small fraction of the whole testis. For these reasons, the hypophysectomized parabiotic rat can, without being sacrificed, serve as a test object for the so-called gametokinetic and luteinizing or interstitial cell-stimulating hormones.

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Our observations are based on 10 pairs of animals of which 9, at the time this article was written were still alive and healthy after 2-13 months of parabiotic life. In most instances the pairs consisted of littermates joined at 50-70 days of age. Most of these pairs went through a preliminary stage during which one partner was a normal rat and the other was hypophysectomized. Finally, however, 8 of the normal partners were castrated, one was made cryptorchid, and one was permitted to remain normal. In 2 cases the castrate was a female.

Normal + Hypophysectomized. In no instance did the normal twin provide enough hypophyseal hormone to prevent eventual atrophy of the testes in the hypophysectomized partner. Sometimes such atrophy was retarded, taking 1-2 weeks longer to occur than that in a non-parabiotic hypophysectomized control where testicular atrophy is markedly evident grossly 15 days after pituitary ablation. During this time, and subsequently, the genital organs of the normal twin remained apparently normal. These findings are similar to those we obtained previously.

Cryptorchid + Hypophysectomized. That a cryptorchid's hypophysis may in some way provide more gonadokinetic hormone than the pituitary of a normal animal was demonstrated in the one pair of this type. At first this pair consisted of a normal male with a hypophysectomized male. Under these conditions the testes of the hypophysectomized test partner underwent rapid atrophy. The normal twin was then made cryptorchid. No evidence of gonadal stimulation was observed in the hypophysectomized partner at first, but 40 days after the normal animal had been rendered cryptorchid, the scrotum of the hypophysectomized twin began to become flushed and edematous, indicating the presence of male hormone. Development of the scrotum and testes continued, but at a very slow rate. Seven weeks after the initial stimulation was noticed, the testes and scrotum of the hypophysectomized twin appeared to be only about ⅓ normal. Throughout this time, the scrotum of the cryptorchid partner remained turgescent and flushed, showing that the abdominal testes were still producing male hormone.

This experiment left no doubt that an animal with abdominally located testes was able to supply a hypophysectomized partner with more gonad-stimulating hormone than the same animal with normal testes. This result lends support to the idea that the pituitary of a cryptorchid animal lies somewhere between those of the normal and the castrate in gonad-stimulating potency,[4, 5, 6] and also indicates the necessity for long-continued observations in parabiotic experiments of this type. In some short-time experiments[1] it was found that cryptorchid and normal partners had about equal effects on the testes of hypophysectomized test animals.

Castrated + Hypophysectomized. In our experience the testes of hypophysectomized rats joined with castrated male or spayed female rats have invariably been maintained normal or, if atrophic at the start, have been returned to a normal condition within a period of 5 weeks. We have been able to confirm our previous findings that the gonadotropic hormone complex passing from a castrated partner will cause in the testes of a hypophysectomized twin parallel stimulation of the interstitial cells and spermatogenic elements. In addition, it has been found that hypophysectomized rats with testes maintained by pituitary gonadotropic secretion from a castrated male or female twin are capable of siring normal litters for at least 6 months following their hypophysectomy. The external genitalia of 2 such hypophysectomized rats are shown in Figs. 1 and 2.

The anterior hypophyses of castrated rats whose hypophysectomized partners receive complete gonadal activation are entirely similar to those of non-parabiotic castrated animals. They show characteristic castration cells, a marked increase in granulated basophils, a decrease in chromophobes and an apparent diminution in the number of eosinophils (Fig. 3).

The gonad stimulation occurring in hypophysectomized rats paired with castrated partners is so clear cut and invariable that it has been possible to employ this reaction as an indicator of hypophyseal gonadokinetic activity in the castrate. Studies in which the inhibiting effect of male and female hormones on the gonad-stimulating potency of the hypophysis has been so demonstrated will be reported elsewhere.

Conclusions. Autopsy data correlated with observations on living animals have established the following points: (1) The testes of hypophysectomized parabiotic rats may be stimulated by hypophyseal secretion from normal, cryptorchid, or castrated partners. (2) Gonadal stimulation in hypophysectomized test animals is greatest when they are joined with castrated partners and least when

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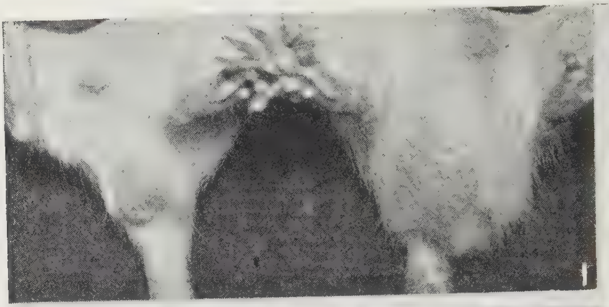

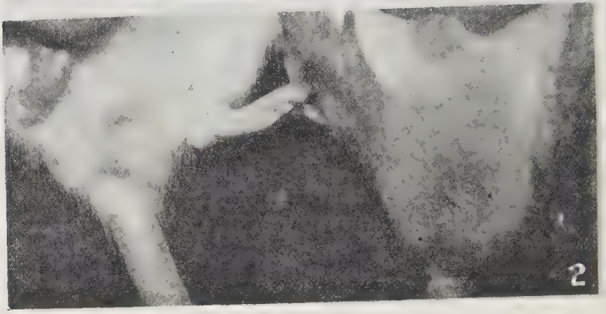

FIG. 1.

Turgescent scrotum containing large testes maintained for a year in a hypophysectomized male rat (right) joined with a castrated male.

FIG. 2.

Same as Fig. 1, except that castrated partner (left) is a female.

FIG. 3.

Portion of anterior pituitary of a male parabiont castrated for 100 days during which time the entire genital tract of a hypophysectomized male twin which had undergone atrophy was completely repaired. Vacuolated basophilic castration cell (V), Granulated basophil (B), Eosinophil (E), Chromophobe (C). Zenker-formol fixation, Mallory trichrome. (× 300.)

they are joined with normal partners. (3) Growth of the scrotum in hypophysectomized parabionts indicates interstitial cell activity, and increase in testicular size reflects predominantly spermatogenic activity. (4) Both these types of activity progress simultaneously under the influence of hypophyseal hormone from a partner, or regress simultaneously in the absence of adequate hormonal stimulation. (5) These results do not support the idea that the castrate hypophysis produces only, or chiefly, gametokinetic hormone, but are in agreement with our previous findings which indicated that the hypophyseal gonadokinetic hormone complex in castrate, cryptorchid and normal parabiotic rats differed only in degree but not in type.

9615 P

Icterus Complicating Pneumonia.
I. Corpuscular Fragility in the Normal Negro.

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The latest editions of medical textbooks make only passing references to the icterus accompanying many cases of lobar pneumonia. Cecil[1] indicates that jaundice occurs in 5 to 10% of cases of lobar pneumonia and that the lighter form which is observed most frequently seems to have no significance while the deeper jaundice seen in other cases is associated with a toxic hepatitis and a grave prognosis. Musser[2] describes the jaundice that is present in a small number of cases of lobar pneumonia as often existing in connection with an enlarged liver and a tender area in the region of the gall bladder. Meakins[3] barely mentions jaundice as a result of metastatic lesions in the liver in lobar pneumonia. Emerson,[4] in a footnote, suggests that this jaundice may be due to the absorption of alveolar exudate or to a slowing of the portal circulation.

During the past year the authors observed 32 cases of lobar pneumonia in adult Negro patients admitted to the medical service of George W. Hubbard Hospital of Meharry Medical College. Twenty-five (78%) of these cases were clinically icteric. This clinical icterus (icteric sclerae, deeply pigmented urine, etc.) was confirmed in the laboratory by icteric index studies. There was no tendency towards tenderness over the liver or gall bladder in these cases and no indications of sufficient interference with portal circulation to account for the icterus. Elton[5] has pointed out that icterus is more marked in the lobar pneumonia of Negro patients than that observed in Whites.

Because of this high incidence of clinical icterus in the Negro the authors felt it advisable to investigate certain aspects of normal

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[4] Emerson, C. P., *A Text Book of Medicine,* 1st edition, J. B. Lippincott, Philadelphia, 1936, p. 30.

[5] Elton, N. W., *J. Lab. and Clin. Med.,* 1931, **17**, 216.

Negro blood to determine whether some property of the red blood corpuscles might account for this difference. The resistance of the red blood corpuscles of the Negro to hypotonic salt solutions as studied in normal Negroes is briefly presented in this paper.

The relatively few workers who have investigated the resistance of the red blood cells to hypotonic salt solutions in cases of pneumonia have reported figures above that of normals. Needles[6] reported figures as low as 0.3% for beginning hemolysis in severe cases. Petrongi[7] found increased resistance of the red cells of children during pneumonia. His normal values were given as 0.36% to 0.44% and were obtained on 100 Brazilians of mixed races. He also suggested the possibility of race and climate playing a part in the difference in resistance of the red cells.

The figures given for the normal fragility of red cells in the standard textbooks of clinical pathology differ only slightly although several methods are used. Thus Todd and Sanford,[8] Cummer,[9] Kolmer and Boerner,[10] Nicholson,[11] and Osgood and Haskins,[12] all give figures which fall within 0.45% NaCl for beginning hemolysis and 0.3% for complete hemolysis. Only Kilduffe[13] gives a range as large as 0.47% for beginning hemolysis and 0.28% for complete hemolysis.

While running a series of fragility tests on patients with pneumonia in which jaundice was a complication, it was noted that many of the normal controls likewise gave figures which indicated increased resistance of their cells to hypotonic salt solutions. These latter findings led to the investigation of fragility test on normal Negroes, the preliminary report of which is given here.

Our series includes 56 rural school children between the ages of 6 and 16, and 38 young adults between the ages of 17 and 23. Careful checks on white and red cell counts, hemoglobin, and temperature showed that these persons were not ill at the time of our tests. Sanford's method as modified by Holloway was used.

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[6] Needles, Robert S., *Arch. Int. Med.*, 1936, **57**, 174.

[7] Petrongi, Gyozo, *Erythrocyte Resistance in Various Stages of Pneumonia* (quoted by above).

[8] Todd and Sanford, *Clinical Diagnosis by Laboratory Methods,* 7th edition, Saunders, Philadelphia, 1931.

[9] Cummer, *Clinical Laboratory Methods,* 3rd edition, Lea and Febiger, Philadelphia, 1931, p. 80.

[10] Kolmer and Boerner, *Approved Laboratory Technique,* D. Appleton & Co., N. Y., 1931, p. 107.

[11] Nicholson, *Laboratory Medicine,* 2nd edition, Lea and Febiger, Philadelphia, 1934, p. 154.

[12] Osgood and Haskins, *Laboratory Diagnosis,* P. Blakiston's Sons Co., Philadelphia, 1931, p. 376.

[13] Kilduffe, *Clinical Laboratory Procedure,* C. V. Mosby Company, St. Louis, 1936, p. 159.

Results. The cells of 72.2% of those tested did not show beginning hemolysis until a solution of 0.4% was reached. In 14.8% of the cases hemolysis did not begin until a solution of 0.36% NaCl was reached. This figure lies within the limits for complete hemolysis as given by several texts. In 22% of the cases complete hemolysis did not occur until a solution of 0.28% was reached.

At present our findings would indicate that the red blood corpuscles of the average Negro are more resistant to hypotonic salt solutions than is indicated for normals in textbooks. It is assumed by the authors that these normal values were probably obtained from the study of average groups of white patients. It is furthermore indicated that the factor of fragility of red blood corpuscles does not account for the high incidence of jaundice observed in lobar pneumonia in the Negro.

9616 P

A Pharmacological Ejaculation Test for Bio-Assay of Male Sex Hormone.

S. LOEWE.

From the Department of Pharmacology, Cornell University Medical College, New York City.

Mammalian tests for the male hormone are preferable to bird tests, a fact which has been stressed by Loewe and Voss since the discovery of the hormone[1, 2, 3] and has gained general recognition through the differentiation of two active principles—testosterone and androsterone (Laqueur,[4] Rucicka[5]). However, mammalian tests in use are unsatisfactory, in that not all of the functions governed by the hormone are observed. This objection does not apply to an ejaculation test since the latter requires the integrity of numerous central and peripheral sex functions. The production of ejacu-

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[1] Loewe, S., and Voss, H. E., Letter to Akad. d. Wiss. Wien, Jan., 1927, published in *Akadem. Anzeiger*, Vienna, 1929, No. 20.

[2] Loewe, S., and Voss, H. E., *Klin. Wochenschr.*, 1930, **9**, 481.

[3] Loewe, S., *Dermatol. Z.*, 1932, **64**, 223.

[4] David, K., Dingemanse, E., Freud, J., and Laqueur, E., *Z. Physiol. Chem.*, 1935, **233**, 281.

[5] Rucicka, L., and Wettstein, A., *Helv. Chim. Acta*, 1935, **18**, 1264.

lation by electrical stimulation has been employed in a procedure for detecting the action of the male hormone on the guinea pig,[6] but it has not been developed into a quantitative method, and it is difficult to elicit the response in the smaller rodents. In the mouse the production of ejaculation by the action of drugs[7] provides an excellent method for studying the ejaculatory function. This method not only detects the action of male hormones, but supplies a means for their bio-assay. The experiments reported here deal with its use in a method of bio-assay.

The experiments were performed on adult albino mice. Ejaculation was elicited by the pernostone-yohimbine technic which will be described in detail elsewhere.[7] This consisted essentially of the hypodermic injection of 83 mg./kg. of pernoston in a 0.67% solution followed in 12 minutes by the subcutaneous injection of 10 mg./kg. of yohimbine sulfate in a 0.5% aqueous solution; 5 minutes later the mouse is brought into a lateral position. Ejaculation occurs within 5 to 40 minutes. Only animals which gave a prompt ejaculatory response in the pernostone-yohimbine test before, but not after castration, were used for the hormone injections. After experimenting with various types of hormone administration, preference was given to a slow method, *i. e.,* one daily injection on 5 successive days. A few series with a rapid method—3 injections within 36 hours—will be briefly mentioned. The animals were repeatedly subjected to the pernostone-yohimbine test at intervals of from 2 to 4 days both during and after the period of hormone injection. Testosterone and testosterone propionate, and in further experiments testosterone acetate and androsterone, were used for the hormone treatment. All substances* were injected in a solution of oil, the volume not exceeding 0.5 cc. The experiments are summarized in Table I.

Ejaculation under the conditions described is an all-or-none indicator of sexual regeneration. The individual response was considered positive when a coagulated ejaculate was perceptible inside or outside of the prepuce with or without magnification. In each group the frequency of response (the ratio number of animals responding to the total number of animals in a group) was taken as a measure of the intensity of action. Tentatively, a frequency of 0.5 (50%) was considered the unit of intensity. The persistence of

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[6] Moore, C. R., and Gallagher, T. F., *Am. J. Physiol.,* 1929, **89**, 388.

[7] Loewe, S., *J. Pharm. and Exp. Therap.,* in press.

* All four substances were kindly provided by Ciba Pharmaceutical Products, Inc., through the courtesy of Dr. E. Oppenheimer.

TABLE I.

Frequency and Persistence of Ejaculatory Response of Castrated Mice after Administration of Varying Hormone Doses Over a Period of 5 Days.

Substance	Daily gamma	Animals Used	Animals Responding	Frequency of Ejaculation %	Persistence Days
Testosterone	12½	8	1	12	5
	25	11	5	45	10
	37	7	3	43	5
	50	5	4	80	10
	100	4	3	75	5
Testosterone propionate	5	8	3	37	> 7
	10	5	2	40	7
	12½	8	4	50	>10
	25	7	6	86	<13
	50	5	5	100	17
	100	6	6	100	>19
	250	4	4	100	?
	500	8	6	75	?
	1000	2	2	100	>27
Testosterone acetate	12½	8	1	13	3
	20	8	4	50	10
	35	8	4	50	> 7
	50	9	8	89	10
	75	9	7	78	>12
	100	4	2	50	11
Androsterone	500	4	0	00	0
	1000	12	5	42	6
	2000	6	2	33	6
	3000	5	3	60	6

action was also measured, being determined by the time interval between the last injection of the hormone and the last ejaculatory response.

As the table shows, the frequency of ejaculation increased with the dose of the hormone. The unit potency of testosterone was found to be about 40 gamma and of the propionate 12.5 gamma. That of the acetate was close to the propionate and that of androsterone about 2500 gamma. These doses are not essentially larger than those required for the mammalian tests in use;[8, 9, 10] the total amount of hormone required is still lower, since the number of injections is smaller. The relative potency (reciprocal ratio of the unit doses) is androsterone : testosterone : propionate = 1 : 62 : 200, *i. e.*, similar to the ratios of about 1 : 20 : 200 found by other mammalian tests and very different from those of about 1 : 5 : 4 found by the capon test.[8, 9, 10] The propionate showed a greater persist-

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[8] Rucicka, A. L., and Wettstein, A., *Helv. Chim. Acta*, 1936, **19**, 1142.

[9] Tschopp, E., *Arch. Internat. de Pharmacodynamie*, 1936, **52**, 391.

[10] Miescher, K., Wettstein, A., and Tschopp, E., *Biochem. J.*, 1936, **30**, 1977.

ence of action than any of the 3 other substances examined. This property of the propionate was still more striking in the small number of short interval (36 hours) experiments. The unit dose was considerably higher under these conditions but the action persisted more than 20 days.

The absolute values obtained in this study may require revision. Various circumstances indicate that they will be found somewhat lower in a larger series of experiments, when the experimental conditions are better established as, for instance, the volume of the hormone solution injected and the time interval between tests on the same animal. However, if the general results of these experiments are confirmed in a larger series of tests, a new bio-assay method becomes available which offers several advantages, *viz.*, animals need not be sacrificed, autopsy and histological examinations or measurements of organs are not required, and the persistence of action can be registered on the individual animal continually.

9617

Blood Copper and Iron in Addison's Disease.

ADOLPH SACHS, VICTOR E. LEVINE AND WILLIAM O. GRIFFITH.
From the School of Medicine, Creighton University, Omaha, Neb.

In a series of anemias previously reported,[1, 2] we have shown that with few exceptions that low blood iron was accompanied by an increase in blood copper. We report now 3 cases of Addison's disease, a comparatively rare condition accompanied by secondary anemia and characterized in most instances by an increased degree of pigmentation of the skin. The fact that the copper content of the skin has recently been closely associated with increased skin pigmentation[3, 4, 5] makes these determinations of added interest.

We have made iron and copper analyses on the blood of 3 patients with Addison's disease. Iron estimations were made by a dry ashing method using potassium thiocyanate as the reagent, and

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[1] Sachs, A., Levine, V. E., and Fabian, A. A., *Arch. Int. Med.*, 1935, **55**, 227.

[2] Sachs, A., Levine, V. E., and Griffith, W. O., PROC. SOC. EXP. BIOL. AND MED., 1936, **35**, 6; 1936, **35**, 332.

[3] Cunningham, I. J., *Biochem. J.*, 1931, **25**, 1267.

[4] Gorter, F. J., *Nature*, 1935, **136**, 185.

[5] Sarata, U., *Jap. J. Med. Sci. II. Biochem.*, 1935, **3**, 79.

copper determinations by a modification of McFarlane's method, using sodium diethyldithiocarbamate as the reagent.[1, 6–9]

TABLE I.

Name of patient	Red Cells per cc. mm.	Hemoglobin, gm. per 100 cc.*	Iron, mg. per 100 cc.	Copper, mg. per 100 cc.	Degree of Pigmentation
Mr. S., 26 yr.	4,370,000	10.47	35.08	0.141	4 plus
Mrs. H., 39 yr.	3,880,000	10.42	34.92	0.224	3 ”
Mrs. C., 38 yr.	3,888,000	10.64	35.64	0.193	1 ”

*Hemoglobin calculated from blood iron by the factor: mg. of Fe per 100 cc. divided by 3.35 = gm. of hemoglobin per 100 cc.[10]

In comparison with our figures obtained for normal individuals, the blood findings in the three patients indicate the presence of anemia. The blood picture in the two female patients shows a hypo-erythrocythemia and hypoferronemia accompanied by hypercupremia. The blood in the male patient reveals a hypo-erythrocytemia and hypoferronemia, the copper content being slightly higher than the average but yet within the normal range.

In the 3 cases of Addison's disease reported in this communication we are unable to deduce any correlation between the copper content of the blood and the amount of skin pigmentation. Mr. S., who possessed the greatest degree of pigmentation of the three, had the least blood copper content. Mrs. C., who displayed the least skin pigmentation, had 37% more blood copper than Mr. S. However, it must be remembered that skin pigmentation in Addison's disease is a very slow and gradual process, and as a result the blood copper may show no deviation from the normal.

We wish to thank Dr. Russel M. Wilder, of the Mayo Clinic, for permission to make copper and iron determinations on blood samples from the 3 patients mentioned in this paper.

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[6] Wong, S. Y., *J. Biol. Chem.*, 1928, **77**, 409.

[7] McFarlane, W. D., *Biochem. J.*, 1932, **26**, 1022.

[8] Sachs, A., Levine, V. E., and Appelsis, A., *Arch. Int. Med.*, 1933, **52**, 366.

[9] Fabian, A. A., Sachs, A., and Levine, V. E., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 662.

[10] Butterfield, E., *Z. f. physiol. Chem.*, 1909, **62**, 173.

9618

Effect of Testosterone Propionate upon Gonadotropic Hormone Excretion and Vaginal Smears of Human Female Castrate.

UDALL J. SALMON.* (Introduced by R. T. Frank.)

From the Department of Laboratories of the Mount Sinai Hospital, New York City.

It has been previously shown that the excessive gonadotropic hormone excretion which occurs after castration in females can be controlled by the administration of adequate amounts of estrogenic substance.[1] Butenandt and Kudzus[2] have shown that testosterone, if given in sufficiently high doses, causes premature opening of the vagina in infantile rats.

The present investigation was undertaken to determine (a) whether the gonadotropic hormone can be made to disappear from the urine of a female castrate by the exhibition of male hormone and (b) whether at the same time an estrogenic effect can be demonstrated in the vaginal smears of the castrated patient.

The patient studied was 46 years of age and had had an hysterectomy and bilateral salpingo-oophorectomy 6 years before the present investigation. During a preliminary study of 20 days (started February 13, 1937) the urine was found to be constantly positive for G. H. (the excretion varying from 13 to 27 R.U. per day) and the vaginal smears were uniformly negative. (Fig. 1.)

The acetone precipitation method was used for G.H. determination in the urine.[3] The vaginal smears were prepared and graded according to the technic and classification previously reported,[4] reactions I and II representing "negative" smears and III and IV "positive" smears.

The patient was given 815 mg. testosterone propionate† in solution in sesame oil in divided doses intramuscularly over a period of 27 days. Individual doses varied from 10 to 50 mg. G.H. deter-

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* Hiram N. Vineberg Research Fellow.

[1] Frank, R. T., and Salmon, U. J., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 311.

[2] Butenandt, A., and Kudzus, H., *Hoppe-Seyler's Z.*, 1935, **237**, 75.

[3] Frank, R. T., Salmon, U. J., and Friedman, R., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 1666.

[4] Salmon, U. J., and Frank, R. T., PROC. SOC. EXP. BIOL. AND MED., 1936, **33**, 612.

† For the testosterone used in this study we are indebted to Dr. Erwin Schwenk of Schering Corporation, Bloomfield, New Jersey.

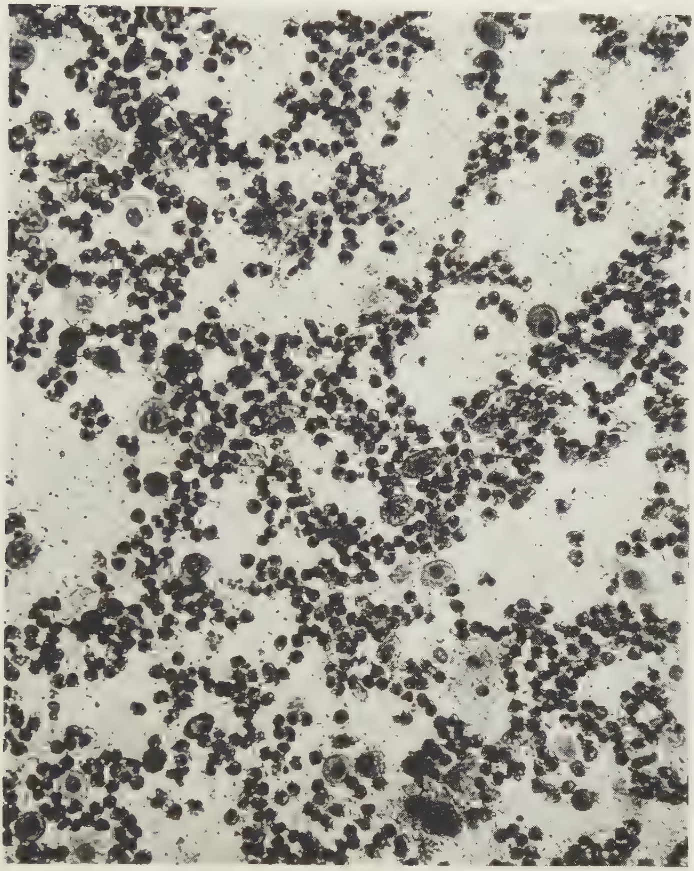


FIG. 1.

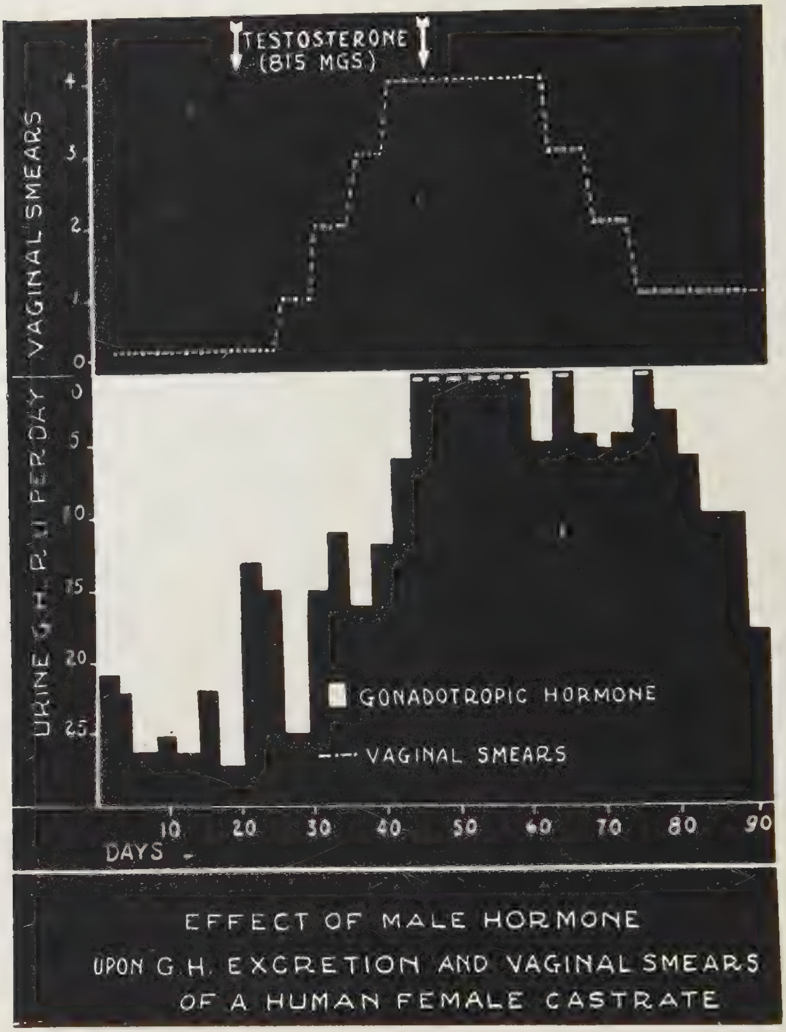
Preliminary "negative" smear exhibiting predominance of leucocytes with scattered, small epithelial cells.

minations and vaginal smears were performed throughout the period of observation of 92 days.

The results are represented in Graph 1.

The G.H. factors began to disappear from the urine after 465 mg. of testosterone had been administered. For a period of 18 days the urine remained negative for G.H. Following this, G.H. reappeared in the urine in increasing concentration. Coincident with the disappearance of G.H. from the urine large epithelial cells which exhibited various degrees of cornification appeared in the vaginal smears displacing completely the "compact cells" (Papanicolaou) and the leucocytes, thus representing a full estrogenic effect. (Fig. 2.)

The maximum effect on the smear lasted for 21 days, following



GRAPH 1.

Note the disappearance of gonadotropic hormone from the urine and the change in the vaginal smear reaction following the administration of testosterone propionate.

which a gradual return to the pre-treatment status occurred. The regression of the smear ran parallel with the reappearance of G.H. in the urine.

It is interesting to note that the patient began to experience relief of her symptoms (flashes, headaches, etc.) after 400 mg. of testosterone propionate had been administered. The amelioration of symptoms reached its maximum at the time when the smear exhib-

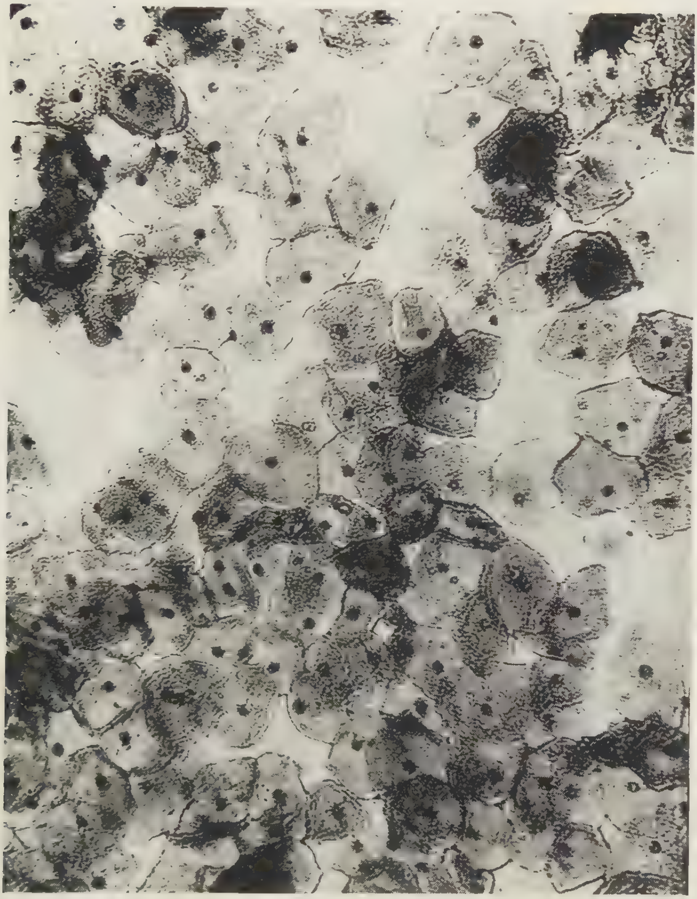


FIG. 2.
Estrogenic effect in vaginal smear following testosterone propionate.

ited a full estrogenic effect and the urine was negative for G.H. Symptoms recurred rapidly with the reappearance of G.H. in the urine and the regression of the smear to its pre-treatment status. Identical effects (as regards both G.H. excretion and vaginal smears) had been produced in the same patient 24 months previously with 16,000 R.U. of estradiol benzoate.

It would appear from this study that testosterone propionate when given in adequate dosage can inhibit the gonadotropic hyperactivity of the hypophysis in a female castrate and also produce a full estrogenic effect on the vaginal mucus membrane of a human castrate—thus paralleling two phases of biologic activity of the estrogenic substances.

9619 P

A Transplantable Spontaneous Bone Tumor in *Mus musculus*.

Arthur M. Cloudman. (Introduced by C. C. Little.)
From Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine.

Bone tumors, chiefly sarcomata, are occasionally observed in our laboratory as spontaneous new growths in some of the inbred stocks of *Mus musculus* and in their hybrids. The following preliminary report is, I believe, the first recorded case of a successfully transplanted spontaneous bone sarcoma in the common house mouse.

On August 25, 1936, virgin female mouse 26316 of the C57 Black stock was killed at the age of 743 days. She had 2 different types of tumors, (1) a bony growth, L946A, about 6 mm. in diameter which partially encircled the tail at about a third of the distance from the body to the tip, and (2) generalized lymphoblastomatosis, called tumor L946B. Tumor L946A was so bony that it

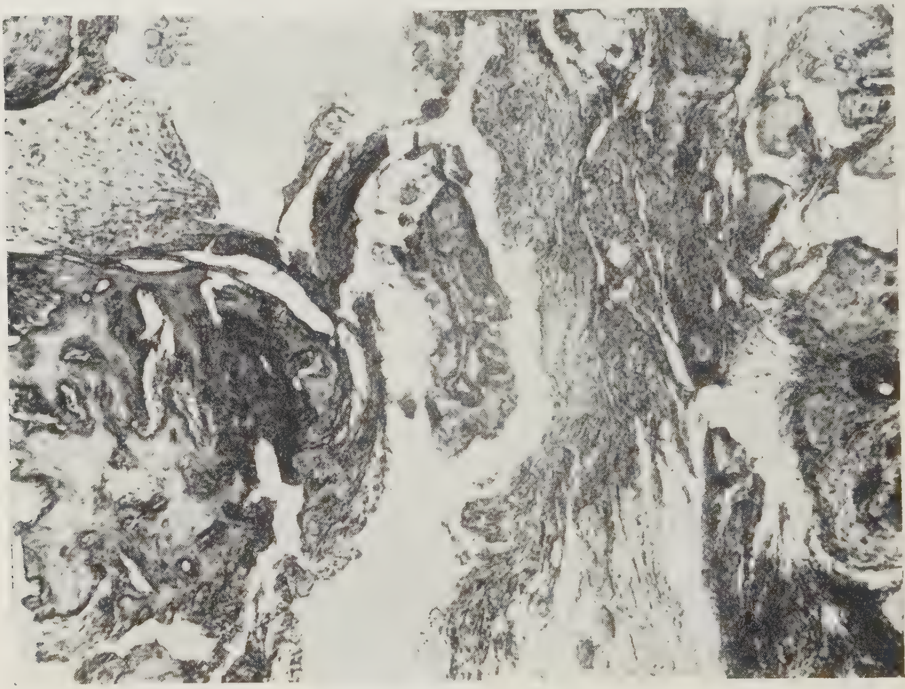


Fig. 1.

Primary osteogenic sarcoma, L946A, from tail vertebra of C57 Black virgin female mouse 26316. Remains of invaded striated muscle seen near right end of lower margin. × 75.

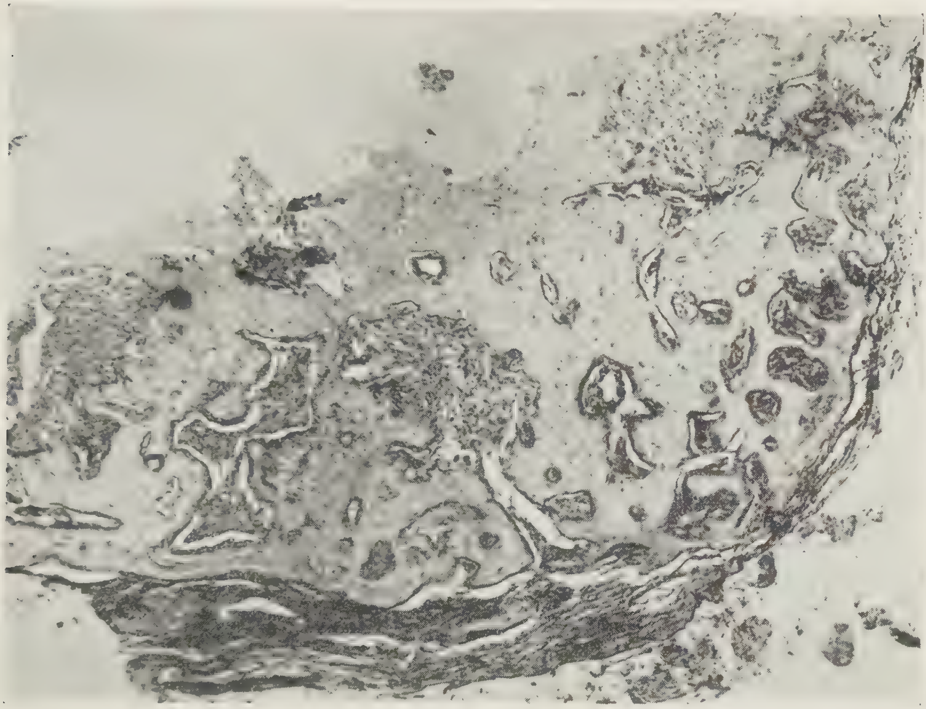


Fig. 2.

Implant I of tumor L946A composed chiefly of dense newly formed bone surrounded by fibrous capsule. × 75.

was broken, rather than cut, into 5 fragments. One piece was saved for sectioning and 4 were implanted subcutaneously into four C57 Black stock males aged 30 days.

These first 4 implants were given the numbers I, II, III and IV on the tumor chart used to record evidence of either their growth or their absorption. For the next 7 weeks these masses were palpated periodically and there was little if any noticeable change except that they became less angular in outline. At 8 weeks 3 of the 4 tumors showed an increase in size. Number II grew the fastest and was somewhat yielding on palpation in contrast to III and IV, which were both very hard, bony and irregular in outline. Number III remained the smaller of the bony growths.

At 14 weeks all 4 mice were killed, autopsied and specimens of all 4 tumors saved for sectioning. Implant I was apparently unchanged in size and consistency. Number II was 2.5 cm. in diameter and on gross section exhibited a fleshy bulging cut surface with only a small central gritty core. Number III was 1 cm. in diameter and had bony spicules scattered throughout the growth. Implant IV was 1.5 cm. in diameter and was composed almost entirely of spongy bone. Small pieces of tissue from growths II, III and IV were transplanted into other mice of the same stock.

On histological examination the primary bony tumor was found to be a slowly growing osteogenic sarcoma which showed beginning invasion of the adjacent muscle (Fig. 1). Implant I was well encapsulated and was more like an osteoma, being more benign in appearance than was the primary growth (Fig. 2). Number II was composed of rapidly dividing spindle cells with a small central focus of osteogenic sarcoma apparently the remnant of the bony transplant. The actively growing part is shown in Fig. 3. Numbers III and IV were osteogenic sarcomata. The former had a moderate amount of newly formed bone while in the latter there was an abundance of newly formed bone and many rapidly dividing spindle cells (Figs. 4 and 5).

In the second transplant generation tumor II averaged between 2

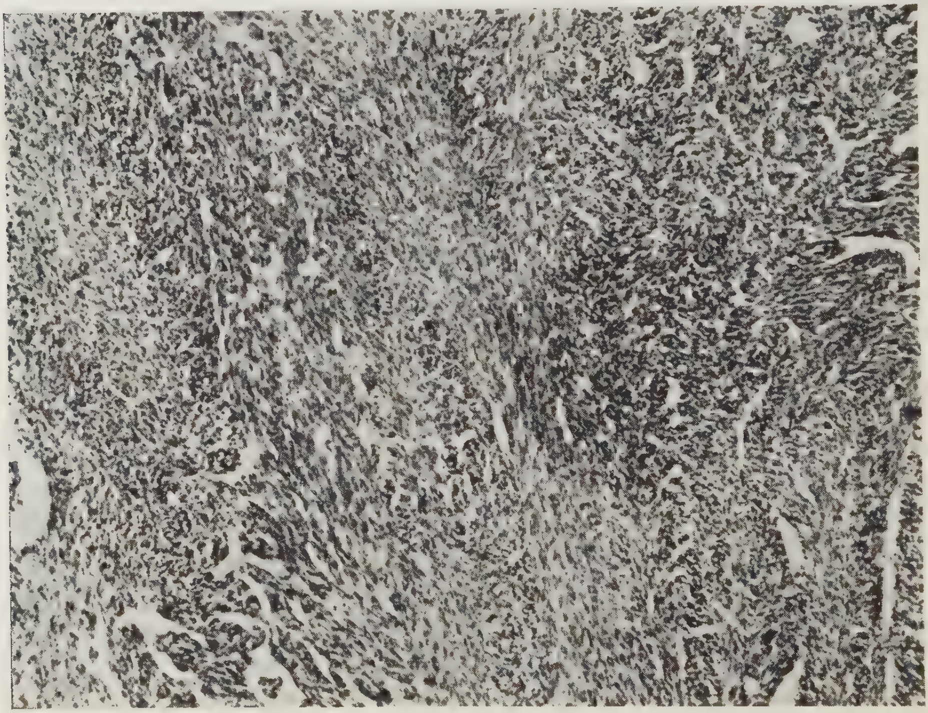


FIG. 3.

Implant II of tumor L946A with the actively growing part composed of rapidly dividing spindle cells. × 75.

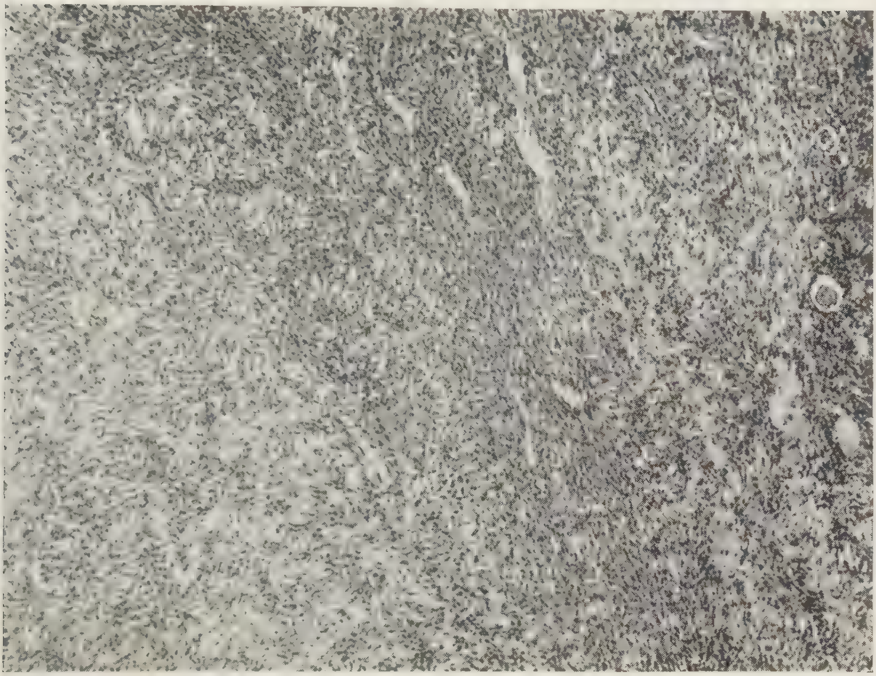


FIG. 4.
Implant III of tumor L946A showing small islands of newly found bone. × 75.

and 3 cm. in diameter at the end of 6 weeks. The growth was rather soft and the skin over it showed a definite tendency to ulcerate. All the animals died soon after this. Histological examination of these tumors showed no bone, but a rapidly growing fibrosarcoma which was invading and destroying the epidermis. At the end of this same period tumor III was about 0.5 cm. and tumor IV was 2 to 3 cm. in diameter. Both these tumors were bony, hard and nodular.

After 8 to 9 weeks of growth, IV weighed as much as its host and at 4 months the tumor masses were between 2 and 3 times as heavy as the mice bearing them. Only in the terminal stages did the skin show ulceration over the tumors. Toward the end the mice became almost unable to move about, yet until they were handicapped by the size of the tumor, their general health appeared nearly normal.

Tumor III grew in much the same manner. However, it took 5½ months to obtain masses which were slightly less than twice the weight of their hosts. This slower bone tumor metastasized to the lungs.

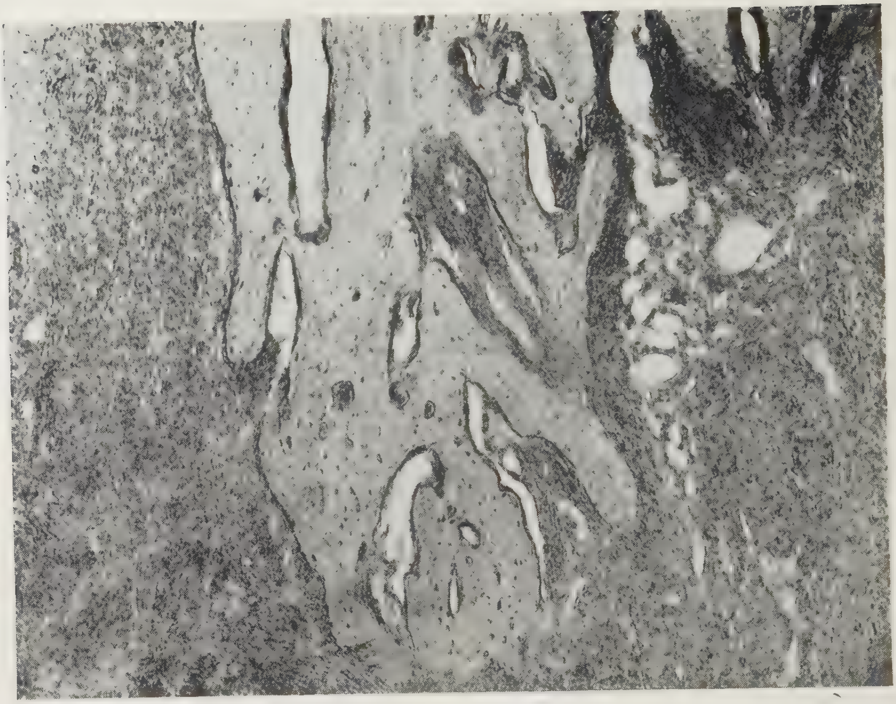


FIG. 5.
Implant IV of tumor L946A with great masses of dense newly formed bone. × 75.

Eleven months after the death of the female in whom the primary bone tumor originated, mass number II was in its ninth, III in its fifth, and IV in its seventh tissue transplant generation.

9620

Nitrogen Retention in a Child During Undernutrition.

ELIZABETH STERN CONN. (Introduced by L. H. Newburgh.)
From the Department of Internal Medicine, University of Michigan, Ann Arbor.

The purpose of this study was to determine if a child could add nitrogen to its body during a period of sharp undernutrition. A review of the literature fails to reveal any similar study. Since addition of protein to the organism is a normal accompaniment of growth, retention of nitrogen is interpreted as evidence of growth.

The subject, a 10½-year-old white female, had been markedly overweight since the age of one year. In several previous admissions to the hospital she had lost weight as a result of controlled underfeeding. At home, however, she always rapidly gained weight. She returned to the hospital in March, 1934. Her weight was 67.97 kg. and height 150 cm. (normal is 41-43 kg.). Except for obesity and a rather dull mentality, examination revealed no abnormalities. The basal metabolic rate varied between 20 and 25% less than the normal on repeated occasions. Laboratory data were otherwise normal.

During the entire period of study the child lived in a special metabolism room where she could be constantly observed. She was weighed carefully each morning on a balance accurate to 2 gm. All food was weighed accurately to 1 gm. immediately preceding each meal. The food was of such a character that waste on the dishes was negligible. We used a constant diet of simple foods,* all of which were analyzed for total nitrogen by the Kjeldahl method. Whenever a new supply of any food was obtained, nitrogen determinations were made. Daily total 24-hourly urine and stool were excreted directly into weighed containers which were kept in the refrigerator during the collections. An aliquot of each 24-hourly collection of urine was analyzed for total nitrogen by the Kjeldahl method. All the feces for each period were dried until constant weight was reached and then analyzed for total nitrogen.

The calorific value of the diet was 600 calories in periods I and III, 800 in period IV, 1140 in period V, and 1740 in period VI (see Table I). The nitrogen content of the diet was kept constant during periods I, III, IV, V, and VI, at about 7 gm. per day. This was done in order to study the effect of varying degrees of undernutrition upon retention of nitrogen. The subject received about one gram of animal protein per kilo of ideal body weight per day. During period II the calorific value of the diet was the same as in period I but the nitrogen intake was increased to 8.35 gm. per day (see Table I).

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* Powdered skimmed milk (Merrell-Soule Co.).
Egg, hard cooked
Whole wheat bread.
Tomato juice.
Grapefruit juice, canned, unsweetened.
Grape jelly.
Washed bran (Cellu).
Butter.
Whole milk.
Cream, 40%.

Changes in the caloric and nitrogen content in the diets of the different periods were accomplished simply by changing the amounts of the above foods.

TABLE I.

Period	Date 1934	No. of Days	Change of Weight in Kg.	Diet			Total gm. per period of Excretory N		Gm. N retained per period	Gm. N retained per day
				Calories per day	Gm. N per day	Total gm. N per period	Urine	Feces		
Prelim.	3/27-4/23	28	— 4.11	600						
I	4/24-5/14	21	— 2.53	600	6.87	144.12	121.10	10.73	12.29	0.54
II	5/15-5/24	10	— 1.36	600	8.35	83.50	72.91	6.98	3.61	0.36
III	5/25-6/6	13	— 0.91	600	7.02	91.22	76.99	8.10	6.13	0.47
IV	6/7 -6/27	21	— 2.69	800	7.14	149.94	130.26	12.63	7.05	0.34
V	6/28-7/9	12	— 0.60	1140	7.09	85.08	69.08	5.71	10.29	0.86
VI	7/10-7/14	8	+ 0.02	1740	7.02	56.16	41.26	5.40	9.50	1.19
Total		113	—12.18						48.87	(Aver.) 0.57

During the entire period of undernutrition there was an average daily retention of 0.5 gm. of nitrogen (periods I through V). Although the retention of nitrogen increased as the calorific value of the diet approached maintenance, there was a significant and constant storage of nitrogen during the periods of the most severe undernutrition. The average of the 44 days, when 600 calories were being fed, (periods I, II, and III) was 0.5 gm. of nitrogen per day. On the other hand, with a constant calorific value of the diet, an increased nitrogen intake (period II) did not increase the nitrogen retained. Over the period of study the child lost 8 kg. of body weight while she retained 49 gm. of nitrogen.

Summary. An obese child, who was sharply underfed for 85 consecutive days, retained a large amount of nitrogen while losing 8 kg. of body weight Although there was a significant and constant retention of nitrogen during the period of most severe undernutrition, the retention of nitrogen gradually increased as the calorific value of the diet approached maintenance. Increasing the nitrogen of the diet did not increase the retention of nitrogen while the subject was being severely underfed.

9621

Relationship Between Vitamin D Intake and Linear Growth in Infants.

Francis Slyker, B. M. Hamil, M. W. Poole, T. B. Cooley and Icie G. Macy.

From the Research Laboratory, Children's Fund of Michigan, and the Children's Hospital of Michigan, Detroit.

Over a 3-year period the Research Laboratory of the Children's Fund of Michigan, cooperating with the Children's Hospital of Michigan, has studied the effect of certain accessory food factors on nutrition, growth and development of infants during the first year of life. Data have been collected which appear to confirm the findings of Stearns, Jeans and Vandecar,[1] who, in an extensive individual study, demonstrated a close relationship between vitamin D intake and linear growth.

Over 500 infants* from urban families of indigent or near-indigent class have been observed. Infants whose family background predisposed to defect and infants presented for initial examination after reaching 10 weeks of age were rejected. The children were observed to the end of their first year of life.

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[1] Stearns, Genevieve, Jeans, P. C., and Vandecar, Verva, *J. Pediat.*, 1936, **9**, 1.

* The expenses of this study were defrayed in part by grants from the Borden Company and the Gerber Products Company.

A carefully balanced artificial diet was prescribed, based on evaporated milk, and the chief elements supplied gratis to the parents.† A daily supply of vitamin C considered adequate was supplied by commercial dehydrated lemon juice powder. Vitamin D was administered in the form of irradiated evaporated milk or standardized cod liver oil.‡ Carbohydrates were added in the form of Karo syrup. Solids were added to the diet at suitable ages.

During monthly visits to the clinic the infants received medical examinations, anthropometric measurements; x-rays of the extremities were taken to be used in appraisal of subclinical rickets, scurvy, or other physical defects. Hemoglobin determinations and red and white blood cell counts were made. Capillary resistance tests for the diagnosis of scurvy (Göthlin's method) were made on 112 of the infants, psychometric examinations on 127. Detailed records were maintained.

Two trained nurses assisted in the clinic and supervised the care of the child in the home. They made frequent visits to the home for this purpose, carrying necessary supplies, and checking the records of feeding and care kept by the mother.

After elimination of all infants who for any reason might militate against "average" quality of the group, 414 infants were eligible for statistical consideration. It was possible to divide the babies into 5 distinct groups on the basis of quantity and source of vitamin D. Racial factors are relatively constant for all groups. We concluded that the averages for our mixed groups are not unduly weighted by either sex, as the various diet groups retain the same relative ranking when the values for boys only are plotted or when the mixed group values are used. Due, however, to the smaller groups thus selected, the curves show greater variability, and for that reason are not presented. The largest group, containing 170 babies, was prescribed cod liver oil in dosages that gave each individual between 250 and 600 U.S.P. units of vitamin D daily. Sixty infants in the second group received an average of 810 units, and a third group of 51 children received 675 units per day. The 76 children in the fourth group received between 95 and 152 U.S.P. units per day from irradiated evaporated milk. Through a milk production error the fifth group of 57 infants received unirradiated evaporated milk until the age of eight lunar months. At this time some of the diagnosticians made an interpretation of rickets from several of the roentgen films.[2] Immediately, therapeutic doses of cod liver oil were prescribed. This corrected any slight rachitic condition before the termination of the study and no effect upon the rate of growth in length could be found.

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† The infants were obtained through the generous cooperation of the Detroit Department of Health, Harper Hospital, Florence Crittenton Hospital, Woman's Hospital, and private physicians in Detroit.

‡ Standardized cod liver oil was kindly furnished for some of the infants by the United States Children's Bureau.

The average heights for each group were calculated for successive 28-day intervals, plotted, and the resulting curves compared by inspection. The curves demonstrated that the average heights of the 3 groups receiving the highest vitamin D intake were almost identical and that the values were noticeably higher than for the 2 groups receiving less vitamin D. Two of these groups received 675 units or more. The other, prescribed 250 to 600 units, undoubtedly received an average amount near to 600 units as the usual procedure was to start each infant at the low dosage and increase it as rapidly as possible to the maximum. Since the 2 groups receiving greater amounts did not increase in length at any greater rate than the third, it would seem that the optimum dosage for growth promotion is very near to 600 U.S.P. units daily.

The 3 groups mentioned included 271 infants who received between 250 and 810 U.S.P. units daily. Averages were obtained for a composite of these 3 groups. These were compared graphically with the averages of the other 2 groups (Chart I). It will be seen that the average heights at the beginning of observation are almost identical for the 3 groups, but thereafter the group receiving the greatest dosage increased most rapidly in length, the group on an intake of 95 to 162 units gained at a noticeably slower rate, and the group supplied with almost no vitamin D gained the slowest. The addition of vitamin D to the regimen of the group at an average age of 225 days apparently failed to increase the rate of growth appreciably, although the occurrence and severity of rickets were diminished.

It has been determined that the lower curve for these 2 groups is not due to the presence in them of infants with moderate rickets. Only 2 cases developed from the 76 in the group receiving 95 to 162 U.S.P. units. Averages for these 2 showed little variation from the averages for the balance of the group. The curve for "moderate" rachitic infants (8) of the "no D" group falls considerably

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[2] Cooley, T. B., and Reynolds, L., *J. Pediat.*, 1937, **10**, 743.

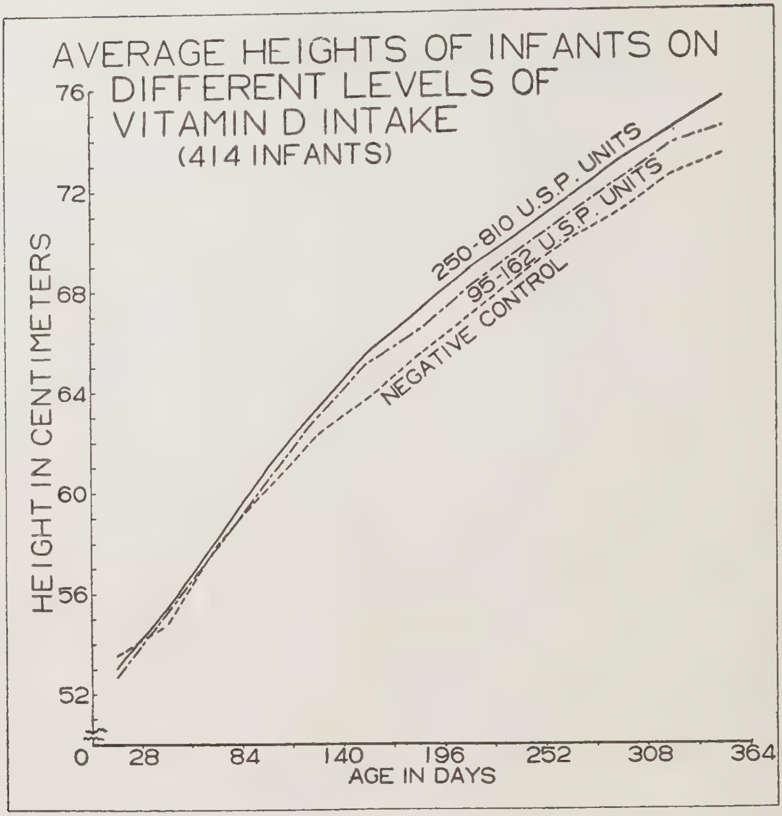

CHART 1.

below that of the remainder but, due to the small proportion of rachitic infants, the curve for the non-rachitic infants is not greatly higher than that of the group as a whole.

The average heights of the infants in this study compare favorably with accepted standards. These comparisons have been published from this laboratory.[3, 4]

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[3] Poole, M. W., Hamil, B. M., Cooley, T. B., and Macy, I. G., *Am. J. Dis. Child.*, 1937, **54**, 726.

[4] Poole, M. W., Hamil, B. M., Cooley, T. B., and Macy, I. G., *Am. J. Dis. Child.*, in press.

9622 P

Homogeneous Heavy Substances from Healthy Tissues.

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From the Rockefeller Institute, Princeton, N. J.

During the course of work with the ultracentrifuge on the virus responsible for "jaundice"[1] in silkworms, macromolecular substances that sediment with the sharp boundaries indicative of a high degree of molecular homogeneity have been isolated from both healthy and diseased worms.

Recent investigations with the analytical ultracentrifuge[2] have demonstrated the sensitivity of viral proteins to salts in moderate concentrations and the consequent importance of using the mildest possible methods of extraction and treatment when dealing with the materials responsible for viral activity. For the present experiments both the fully diseased and the healthy control worms were preserved until needed in the freezing compartment of an electric refrigerator. Several of the frozen worms—usually 15 to 25 in number—were ground with salt in an iced mortar while in this state. A suspension of this mass in 0.05M saline was filtered through hardened filter paper and clarified first in an ordinary centrifuge and then by being put in the quantity-ultracentrifuge and run up quickly to 9000 r.p.m. and immediately stopped. When the dark but essentially clear solution that resulted was ultracentrifuged for an hour in a field of *ca.* 60,000g, a large, dark pellet was obtained which could be partially resuspended in dilute saline and then purified by successive sedimentations and low-speed centrifugations. After 2 or 3 ultracentrifugations the supernate became colorless and the pellet resuspended to give a yellowish, opalescent solution. The ultracentrifugal diagram prepared by the absorption-method from such a solution is shown in Fig. 1. The diagrams from diseased worms have been somewhat poorer and more diffuse than those from healthy worms but the measured sedimentation-constants have been the same in each case. The purified solution from normal worms does not produce observable symptoms of disease when fed undiluted to healthy worms.

It is interesting to compare the properties of these macromolecules from healthy tissues with those of the various plant and animal

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[1] See Glaser, R. W., Virus Diseases of Insects, in *Filterable Viruses,* edited by Thomas Rivers, Baltimore, 1928.

[2] Wyckoff, Ralph W. G., *J. Biol. Chem.,* 1937, **121**, 215.

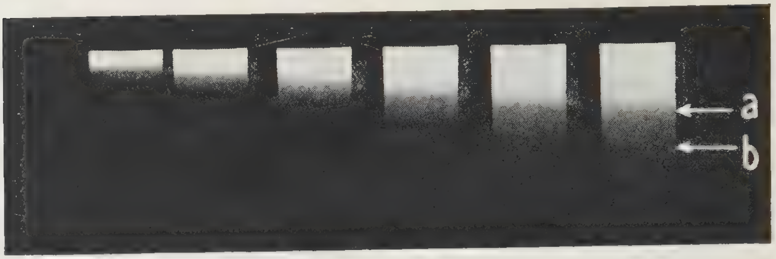

FIG. 1.

Sedimentation-diagram of a macromolecular solution obtained from healthy silkworms. The boundary (a) sediments with $s_{20}° = 53 \times 10^{-13}$, (b) with $s_{20}° = 92 \times 10^{-13}$. Interval between exposures = 5 min. Light source = mercury arc. Filters: bromine plus chlorine cells.

virus-proteins that have been isolated. The healthy substances are unstable and after standing for a short time in the ice-box yield diffuse instead of sharp sedimentation-diagrams; after a couple of days, evidence can no longer be found for large molecules. They are thus less stable than many viral proteins but do not break up more readily than numerous known viruses. Solutions give protein-reactions and absorb strongly in the ultraviolet region below 2700Å, as do proteins. Their analytical sedimentation-boundaries are as sharp as those of viral proteins. These boundaries move with rates that are somewhat smaller but of the same general magnitude as those of the measured viral proteins. Observations on a dozen concordant diagrams (Fig. 1) show that the sedimentative rates are the same for the corresponding substances from healthy and "jaundiced" worms. The measured constants are $s_{20}° = 53 \times 10^{-13}$ and $s_{20}° = 92 \times 10^{-13}$ cm. sec.$^{-1}$ dynes^{-1}. Since these may not be molecules of simple proteins, their weight cannot safely be estimated without determinations of density. Unconjugated proteins sedimenting at these rates would be expected[3] to have molecular weights in the neighborhood of 3 and 6 millions respectively.

Such non-pathogenic macromolecules as these are probably of widespread occurrence. They have already been seen with the ultracentrifuge in extracts of both healthy tissues and tissues inoculated with equine encephalomyelitis[4] and other viruses.

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[3] Svedberg, T., *Chem. Rev.*, 1937, **20**, 81.

[4] Wyckoff, Ralph W. G., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 771.

9623 P

The "Fat Sparing" Action of Glucose in the Absence of Insulin.*

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It is generally acknowledged that the ketone bodies are intermediary products of fatty acid oxidation in the liver.[1, 2, 3] Hence, it is obvious that any procedure which results in a decrease of ketone body production must act by decreasing the rate at which fat is undergoing oxidation, *i. e.,* by sparing fat.

In a series of experiments[4] subsequently confirmed by Barker and Sweet,[5] we demonstrated that when the fasting depancreatized dog is treated with glucose intravenously in amounts adequate for glycogen deposition in the liver, a decrease in the ketone body content of the blood occurs even in the complete absence of insulin. Since it has been shown that this effect of glucose is not due to either an increased utilization[5] or an increased excretion of the ketone bodies,[4] it must be attributed to a decreased formation of these substances, or, in other words, to a decrease in fatty acid oxidation.

In a more recent report, Lichtman[6] demonstrated that while the administration of glucose *per os* results in a decrease in the blood fatty acids of normal dogs, it fails to produce this effect in animals deprived of their pancreas. From these observations, he draws the implication that glucose is "fat-sparing" only when insulin is present in the organism. If a decrease in the blood-fat level were a proper index of "fat-sparing", then when an inhibition of ketone body formation occurs in consequence of the intravenous administration of glucose, a diminution in the blood fatty acids should occur also. It became of interest to study this question.

Dogs were depancreatized, and in order to hasten the onset and aggravate the severity of the ketosis,[4] one gram of phlorhizin in oil and one gram of phlorhizin in sodium carbonate solution were administered subcutaneously 24 hours after pancreatectomy. After

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* Aided by the David May Memorial Fund.

[1] Quastel, J. H., and Wheatley, A. H. M., *Biochem. J.*, 1933, **27**, 1753.

[2] Snapper, J., and Grunbaum, A., *Biochem. Z.*, 1927, **185**, 228.

[3] Mirsky, I. A., and Broh-Kahn, R. H., *Am. J. Physiol.*, 1937, **119**, 734.

[4] Mirsky, I. A., Heiman, J. D., and Broh-Kahn, R. H., *Am. J. Physiol.*, 1937, **118**, 290.

[5] Barker, S. B., and Sweet, J. E., *Science*, 1937, **86**, 270.

[6] Lichtman, A. L., *J. Biol. Chem.*, 1937, **120**, 35.

another interval of from 24 to 48 hours, the animals were anesthetized with sodium amytal† and a branch of the femoral artery was exposed. Two blood samples were drawn at hourly intervals, and then 2 gm. of glucose per kilo of body weight were administered intravenously every hour in a 30% solution. Blood samples were again drawn 2 and 4 hours after the onset of the glucose injections. The "total" ketone body content of the blood was determined by the Van Slyke and Fitz method,[7] and the total fatty acid content of the whole blood by Street's method.[8] As a control, glucose was administered to anesthetized normal dogs in the postabsorptive state, and the blood fatty acids were likewise determined.

TABLE I.
Influence of Intravenous Glucose Administration on Blood Fatty Acids and Blood "Total Ketones."

		Blood "Total Ketones" (Average)			Whole Blood Fatty Acids* (Average)		
Experiment	Dog	0 mg. %	2 hr. mg. %	4 hr. mg. %	0 mg. %	2 hr. mg. %	4 hr. mg. %
Normal	1	—	—	—	462	379	411
	2	—	—	—	565	509	536
	3	—	—	—	724	660	520
Depancreatized	1	19.9	10.4	7.2	489	491	553
	2	20.7	5.0	0.5	879	795	794
	3	38.0	20.3	0.0	924	959	918

* Total Lipids—Total Cholesterol.

Our results (Table I) are in accord with those of Lichtman in that a decrease in the blood fats occurs in the normal dog, following the administration of glucose and that no decrease occurs in the depancreatized animals. However, in all instances, the intravenous injection of glucose to depancreatized dogs, produces a marked fall in the blood ketone bodies. In view of the antiketogenic effect, it is obvious that a sparing of fat does occur in these animals even though simultaneously their blood fat level remains unchanged. Hence, it is probable that the response of the blood fatty acids to glucose administration in the presence of the pancreas is an indication of some effect on fat mobilization rather than on fat oxidation.

Conclusion. The intravenous administration of glucose to depancreatized dogs results in a "fat-sparing" action even in the absence of insulin. This is not associated with a decrease in the blood fatty acids. The action of glucose in depressing the level of the blood fats of normal dogs is attributed to some effect upon fat mobilization which is dependent upon the presence of a normal pancreas.

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† We are indebted to the Eli Lilly Co. for generous supplies of sodium amytal.

[7] Van Slyke, D. D., and Fitz, R., *J. Biol. Chem.*, 1917, **32**, 495.

[8] Street, H. R., *J. Biol. Chem.*, 1936, **116**, 25.

9624

Red Cell and Reticulocyte Counts in Guinea Pigs Following Exposure to Low Pressures.

ALBERT S. GORDON AND WILLIAM KLEINBERG. (Introduced by Harry A. Charipper.)

From Washington Square College, New York University.

Young adult guinea pigs (wgt. 350-400 gm.) were subjected for continuous periods, varying from 5 to 14 days, to pressures of 370-380 mm. Hg. in a specially made low pressure chamber (for details of the chamber, see Dubin[1]). This exposure is sufficient to cause an increase in red cell count of one to 2.5 million per cu. mm., and a reticulocyte count of 6% to 14%. Red cell counts were made from samples of blood drawn from the ear. The cells on 400 squares of a Levy-Hausser chamber were counted by each of us, and we were required to agree within 4%. Reticulocytes stained with brilliant cresyl blue were counted in wet smears according to the method described by Ramsey and Warren.[2] The behavior of the counts, following termination of the stimulus, in 4 representative animals exposed to the low pressures for different periods of time, is shown in Table I. The red cells are given in millions per cu. mm. and the reticulocytes as a percentage of the total reds.

The results obtained from experiments performed on 27 animals may be stated as follows. Soon after termination of the stimulus the counts begin to fall. The red cell counts attain normal values in 20-24 days and the reticulocytes in about 4 to 6 days. The red cell counts do not remain at this normal level but continue to drop, an anemia developing which reaches its maximum approximately a month after removal of the animals from the chamber. This anemia is similar to the one reported by Tyler and Baldwin[3] in rats after exposure to low oxygen tensions. Accompanying this drop below

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[1] Dubin, M., *Quart. J. Exp. Physiol.*, 1934, **24**, 31.

[2] Ramsey, R., and Warren, C. O., *Quart. J. Exp. Physiol.*, 1932, **22**, 49.

[3] Tyler, D. B., and Baldwin, F. M., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 823.

TABLE I.

	59 6 days at 380 mm. Hg.		61 10 days at 380 mm. Hg.		62 10 days at 380 mm. Hg.		—56 14 days at 380 mm. Hg.	
	Reds	Retics.	Reds	Retics.	Reds	Retics.	Reds	Retics.
Normal Count	5.0	0.8	5.6	0.1	5.2	1.2	5.4	0.2
Days Out of Chamber								
0	6.3	6.5	7.0	8.4	6.8	8.6	7.6	8.2
2	6.2	2.0	6.6	3.3	6.6	3.4	7.5	4.3
4	6.0	0.2	6.5	0.4	6.5	0.4	7.3	0.2
8	5.8	0.4	6.3	0.2	6.3	0.1	6.8	0.1
12	5.5	0.2	6.1	0.1	6.0	0.1	6.5	0.2
16	5.3	0.1	5.9	0.1	5.8	0.2	6.0	0.1
20	5.1	0.1	5.8	0.2	5.6	0.1	5.7	0.1
24	4.9	0.3	5.5	0.1	5.2	0.4	5.2	0.3
28	4.6	1.0	5.2	0.2	4.8	1.6	4.5	1.6
32	4.6	1.2	4.8	0.8	4.6	1.9	3.8	8.2
38	5.1	1.2	4.6	2.2	4.8	0.9	4.4	1.2
44	5.0	1.8	5.0	1.6	5.1	0.5	4.8	1.3
50	5.4	1.0	5.1	1.2	5.4	0.4	5.1	0.8
56	5.6	0.5	5.3	1.0	5.6	0.5	5.0	1.0
62	—	—	5.8	0.6	5.8	0.2	5.5	0.6
70	5.6	0.4	6.2	0.9	5.6	0.5	5.3	0.5
82	5.4	0.4	6.1	1.2	5.4	0.2	5.7	0.4
101	5.0	0.3	5.8	0.6	5.2	0.2	5.9	1.0
120	4.9	0.4	5.4	0.4	5.0	0.8	5.2	0.2
142	5.2	0.4	5.5	0.2	5.0	0.2	5.5	0.1
150	5.2	0.2	5.5	0.2	5.3	0.1	5.4	0.2

0 days out of chamber refers to counts taken immediately after termination of the entire low pressure stimulus.

the normal count, there occurs an increase in the reticulocyte count which reaches a maximum at the time when the anemia is most intense. This would seem to indicate that the anemia occurs mainly because of excess destruction of red cells, especially in those cases where the period of exposure is greater than 10 days. The extent of this anemia depends on two factors: (1) the period of exposure to low pressures—in general, longer exposures produce greater rises in red cell count followed by greater percentage drops after withdrawal of the stimulus; (2) the magnitude of the normal red cell count—in animals with lower normal counts, the percentage increases in red cell count, for the same period of low pressure exposure, are almost invariably greater than in animals with higher normal counts. In the former, however, the percentage drops below the normal count of the animal, after termination of the stimulus, are always smaller than in the latter.

Following the anemia, the red cell counts rise, and within another month, pass the normal count and reach a level above it, which is not as high as the one attained originally. As the red cell counts increase, the reticulocyte percentages fall. These excursions of the red cell count above and below normal are still evident 3 months after cessation of the stimulus. They become less and less marked, however, until shortly after the fourth or fifth month, the normal count or a figure near this, is maintained.

The results of this study shed further light on the delicate nature of what Krumbhaar[4] has called the hemolyto-poietic equilibrium. It demonstrates that both the blood-forming and blood-destroying sides of the system possess, when stimulated excessively, a momentum which carries them beyond normal limits, this momentum becoming less and less marked, in pendulum-like fashion, as the stimulus (in this case, either more or fewer cells than normal) becomes less intense.

9625

Sensitization and Antibody Formation after Injection of Tubercle Bacilli and Paraffin Oil.*

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From the Department of Pathology, Cornell University Medical College.

The synergistic effect of various substances upon immunization was noted a long time ago; the literature has been admirably reviewed by Swift and Schultz.[1] The experiments of Dochez[2] have direct bearing on our problem. He succeeded in promoting immunization by the incorporation of streptococci into agar and production of persistent foci. Coulaud[3] sensitized 2 rabbits by the injection of melted paraffin containing killed tubercle bacilli. Ramon and others[4] and Eisler and Gottdenker[5] found that adding paraffin oil to diphtheric toxin accelerates antitoxin-production. Saenz[6] discovered that one cc. of paraffin oil containing killed and dried tubercle bacilli injected into the subcutaneous tissue of guinea pigs produces a very

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[4] Krumbhaar, E. B., *Cowdry's Special Cytology,* 2nd edition, 1932, Vol. 2, Ch. XV.

* This study was aided by a grant from the International Health Division of the Rockefeller Foundation.

[1] Swift, F. H., and Schultz, M. P., *J. Exp. Med.,* 1936, **63**, 703.

[2] Dochez, A. R., *Harvey Lectures,* 1924-25, 131.

[3] Coulaud, E., *Rev. de la Tuberculose,* 1934, **2**, 850.

[4] Ramon, G., Lemetayer, E., and Richou, R., *Rev. d'Immun.,* 1937, **3**, 205.

[5] Eisler, M., and Gottdenker, F., *Z. Immunitätsforsch.,* 1937, **90**, 427; **91**, 49.

[6] Saenz, A., *Compt. rend. Soc. Biol.,* 1935, **120**, 870, 1050.

intense and lasting sensitiveness to tuberculin. He[7] found that in animals so treated the dissemination of living tubercle bacilli from the subcutaneous tissue through the draining lymph nodes is slower than in guinea pigs immunized with a saline suspension of killed tubercle bacilli. Guinea pigs immunized with paraffin-oil suspensions, however, were no more resistant to infection with tubercle bacilli than untreated controls.[8] Hensel[9] reported that when 5 mg. of killed tubercle bacilli incorporated in one cc. of lanolin or vaseline are injected into guinea pigs, very intense sensitization develops and persists for at least one year. Guinea pigs treated with saline suspension become sensitive also, but their sensitiveness is slight and transient.

We injected 8 guinea pigs subcutaneously with 2 cc. of paraffin oil containing 10 mg. of tubercle bacilli (dry weight), in doses undivided or divided into from 4 to 10 simultaneous injections, and tested them for hypersensitiveness and antibodies. One-tenth cc. of 10% tuberculin was used. The tuberculin-reactions were graded according to the largest diameter over the areas of edema : from 0 to 4 mm. = 0; from 5 to 9 mm. = 1 plus; from 10 to 19 mm. = 2 plus; from 20 to 29 mm. = 3 plus; from 30 to 39 mm. = 4 plus; from 40 to 49 mm. = 5 plus. When the area of swelling was ill-defined, one-half was deducted from the grade. In the titration of complement-fixing antibodies, heat-killed tubercle bacilli were used as antigen; the technic was described previously.[10] All animals developed intense and persistent sensitiveness. Necrotic tuberculin-reactions occurred as shown in Table I.

TABLE I.

After the injection of tubercle bacilli Months	No. of guinea pigs	
	With necrosis	With no necrosis
3	1	7
6	4	3
11	5	3
16	1	1
17	0	2
18	1	1

Of 3 animals immunized with killed tubercle bacilli suspended in saline solution instead of oil, one reacted with necrosis once; the other 2 pigs reacted slightly, if at all, in tests performed at intervals of 2 weeks during 2 months. In these 3 animals serum-tests were not made.

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[7] Saenz, A., *Ibid.*, 1937, **124**, 1161.

[8] Saenz, A., *Ibid.*, 1937, **125**, 495.

[9] Hensel, G., *Beitr. z. Klin. Tuberk.*, 1937, **90**, 387.

[10] Freund, J., Laidlaw, E. H., and Mansfield, J. S., *J. Exp. Med.*, 1936, **64**, 573.

In guinea pigs injected with killed tubercle bacilli suspended in paraffin oil, the first antibody-titrations were carried out 11½ months after immunization. The high titers found at that time did not decrease during the following 8 months (see composite graph, Fig. 1).

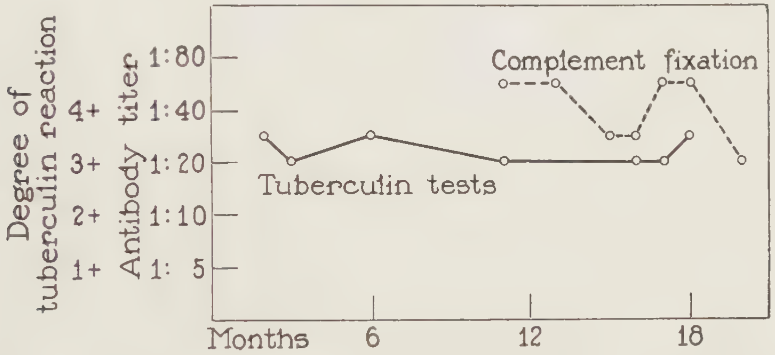

FIG. 1.

Tuberculin reactions and antibody titers in guinea pigs injected with heat-killed tubercle bacilli and paraffin oil.

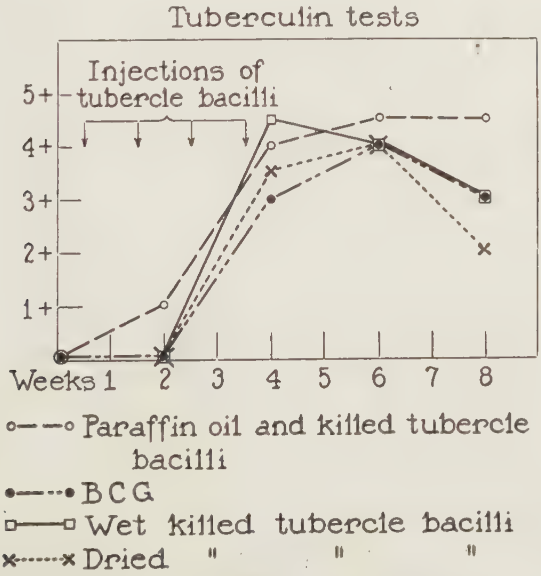

FIG. 2.

Tuberculin reactions in groups of rabbits injected with killed bacilli suspended in paraffin oil, or salt solution, and in rabbits injected with BCG.

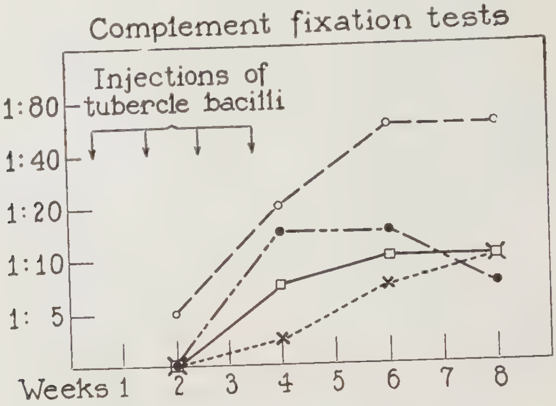

Fig. 3.
Antibody titers in rabbits of Fig. 2.

Guinea pigs treated with oil alone or not treated at all did not react to tuberculin; nor did their sera fix complement.

Tuberculin-sensitiveness and antibody-formation were studied in 30 rabbits immunized with killed and dried tubercle bacilli suspended in paraffin oil, in 9 immunized with killed and dried bacilli suspended in saline, in 9 immunized with killed and undried bacilli suspended in saline, and in 18 immunized with living BCG also suspended in saline. The animals received 4 simultaneous injections of 0.025 mg. of dried tubercle bacilli (equivalent to 0.1 mg. of wet bacilli) in 0.1 cc. of oil or salt solution, or 0.1 mg. of BCG in salt solution, into the subcutaneous tissue at weekly intervals for 4 weeks. Tests for hypersensitiveness and antibody were made 2 weeks apart. (Unlike guinea pigs, rabbits were injected with 0.2 cc. of tuberculin diluted one in 5.)

In the composite graphs the first 3 tests do not reveal appreciable differences in the intensity of reactions to tuberculin. It should be noted, however, that necrosis was frequent in the oil-group and exceptional in the others. The last tests showed that 4 weeks after the last injection of tubercle bacilli, sensitiveness was fully maintained in the oil-group and diminished in all other groups.

The composite graphs of antibody-formation show that paraffin strikingly promotes this function. Rabbits treated with a saline suspension of BCG seem to produce antibodies somewhat more readily than those treated with killed tubercle bacilli suspended in saline. The graphs also suggest that drying of killed tubercle bacilli is harmful to their capacity to produce sensitization and formation of antibody. Comparison of results with BCG and killed tubercle bacilli is uncertain because results with BCG vary greatly in different experiments.

Our results show that the addition of paraffin oil to killed tubercle bacilli augments sensitization and antibody-formation in both guinea pigs and rabbits. In guinea pigs, intense sensitization and antibody formation are astonishingly persistent after the injection of paraffin oil containing killed tubercle bacilli.

The synergistic effect of paraffin oil is probably connected with the nature of the cellular reaction produced by tubercle bacilli coated with an inert oil. (As to the surface-properties of tubercle bacilli in relation to oil and water, see Mudd and Mudd, Mudd and Nugent.[11]) Paraffin oil alone causes only a transient cellular response. The addition of a small amount of paraffin oil (0.02 cc.) intensifies the reaction to killed tubercle bacilli very conspicuously and makes it very persistent. (In a node removed from a guinea pig one year after the injection, large numbers of acid-fast bacilli were found.) Indeed, the local lesions are so extensive that it seems very desirable to modify the preparation of the suspension so that the excess of the amount of oil in relation to the bacteria be reduced. Approximately 20 mg. of killed tubercle bacilli were triturated in an agate mortar alone, later with one drop of oil, and then after addition of saline. The suspension that was formed contained oil droplets up to a few microns in diameter; these are loaded with tubercle bacilli. Immunization employing such a suspension is in progress.

Conclusions. 1. The addition of paraffin oil to killed tubercle bacilli promotes sensitization and antibody-formation in both guinea pigs and rabbits. 2. In rabbits, killed tubercle bacilli incorporated in paraffin oil produce sensitization and antibody-formation with greater intensity than BCG. 3. Twenty months after the injection of killed tubercle bacilli in paraffin oil into guinea pigs, evidence of sensitization still persists and complement-fixing antibody is present in the serum.

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[11] Mudd, S., and Mudd, E. B. H., *J. Exp. Med.*, 1924, **50**, 647; 1926, **53**, 127; Mudd, S., Nugent, R. L., and Bullock, L. T., *Colloid Symposium Monograph*, 1931, **9**, 229.

9626

Relation of Nicotinic Acid to Growth and Dermatitis Factors in Rice Polishings.

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(Introduced by L. Reiner.)

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Various extracts, adsorption and elution products of rice polishings and certain derivatives from yeast were investigated for the growth and dermatitis factors associated in nature with thiamin and riboflavin. These preparations from plant sources were tested with albino rats maintained on a purified sucrose diet,[1] supplemented daily with 20γ thiamin chloride (Merck) and 15γ riboflavin (Labco) and which exhibited cessation of growth and incipient symptoms of the nutritional deficiency.

The biological properties of these concentrates were standardized with growth rates, and curative responses established by depleted animals which received an aqueous acetone extract of rice polishings.* This preparation supplied 6 I.U. of vitamin B_1 in a daily dose of 75 mg. and biological assay or fluorescent examination proved it to be practically free of riboflavin although it is a satisfactory source of the other accessory factors in this group. Growth rates of 1.5-2.0 gm. daily over a period of 4 weeks were employed as a sensitive growth range and were preferred to higher rates which required a maximal response from the animals. Increased amounts of riboflavin (30γ level) have no significant effect on the growth rates which were similar to those obtained with 15γ daily.

An 80% butyl alcohol extraction of the rice polishings concentrate* conducted at room temperature yielded a 3-fold concentration of the essential growth factors, *i. e.,* 25 mg. of the viscous syrup (sp. g. = 1.4) were equivalent to 72 mg. of the rice polishings concentrate. Under similar conditions, a 75% dioxan extraction was more effective than a 90% dioxan extraction and the extractives equalled the butyl alcohol fraction in potency.

A dilute solution of the rice polishings concentrate was treated several times at pH 5 with Surrey fuller's earth. The primary adsorption removed thiamin and associated substances that possessed high adsorption coefficients and that equalled in weight the combined substances adsorbed in the 3 subsequent treatments. The first adsorption product (B_1) was separated from the secondary adsorbates (II) and these latter fractions were combined and eluted with an alcoholic pyridine solution.[2] The pyridine was removed by repeated distillations in a partial vacuum and 20 mg. of the resultant hygroscopic brown powder was found to induce an average rate of growth (1.6 gm. per day) in depleted rats. The lesions of the florid dermatitis in rats[3] were healed with 20 mg. and 50 mg. daily of this material but the animals failed to regain a completely healthy appearance and their rate of growth was only 1.2 gm. per day. This was in marked contrast to the more rapid recovery observed with the butyl alcohol extract or the rice polishings concentrate. In no case where gross lesions had developed did the animals recover with the rapid growth which was typical of rats without dermatitis whose weight had been nearly constant for 10 days at the plateau period. In such animals, realimentation with the rice polishings concentrate produced a growth response of 2.3-2.6 gm. per day. The deficiency appeared more serious than when flavin was lacking and respiratory disorders were common in the diseased rats.

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[1] Cook, C. A., Clarke, M. F., and Light, A. E., *Science,* 1937, **85**, 503.

* "Ryzamin-B," Burroughs Wellcome Co. (U. S. A.) Inc.

A primary adsorbate comparable to the International Standard (B_1) allowed a limited growth at 60 mg. levels when supplemented with riboflavin. The experimental animals required 38 days to exhibit a growth plateau in comparison with controls which received only thiamin chloride and riboflavin and displayed a characteristic plateau in 20-25 days (Table I). An alcoholic pyridine extract of this product at 12 mg. and 24 mg. levels provided similar evidence that a small amount of growth substances other than thiamin and riboflavin were present.

The filtrate from the multiple adsorptions proved to be inactive in 70 mg. doses when administered with only thiamin and riboflavin. Nevertheless, the findings of Lepkovsky, Jukes and Krause[4] were confirmed in that additions of 70 mg. of the viscous filtrate to the 20 mg. dose of the pyridine eluate previously assayed did produce a supplementary effect with a higher rate (2.2 gm. per day) of growth. The adsorption method did not completely separate the so-called filtrate factor and the silicates appeared to adsorb all factors to some extent.

An extraction of autoclaved 'Ryzamin-B' with 80% acetone pre-

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[2] Cook, C. A., and Carroll, R., *Ind. and Eng. Chem.*, 1936, **28**, 741.

[3] György, P., *Biochem. J.*, 1935, **29**, 741.

[4] Lepkovsky, S., Jukes, T. H., and Krause, M. E., *J. Biol. Chem.*, 1936, **115**, 557.

TABLE I.

Preparation	Growth g./day	Assay Period days
20γ Thiamin chloride + 15γ or 30γ Riboflavin	*Plateau	Assays begin at 25th day
72 mg. Rice polishings concentrate	2.3	28
90 ” ” ” ”	†2.6	28
5-10 mg. 80% Butyl alcohol extract	0.8	18
20 ” ” ” ” ”	1.6	30
25 ” ” ” ” ”	2.0	30
50 ” ” ” ” ”	†2.2	21
50 ” 80% Acetone extract of autoclaved rice polishings concentrate	0.8	24
20 ” Pyridine eluate of adsorbates II	1.6	14
20 ” ” ” ” ” ”	1.2	21
50 ” ” ” ” ” ”	‡1.2	35
20 ” Autoclaved pyridine eluate of adsorbates II	0.9	14
70 ” Filtrate from II	no growth	14
70 ” ” ” ” + 20 mg. eluate of II	2.2	28
70γ, 140γ, or 1 mg. Nicotinic acid or amide	§no growth	
5 mg. Adenylic acid + 1 mg. nicotinic acid amide	” ”	
25 ” Rice polishings concentrate	1.2	7
25 ” ” ” ” + 70γ — 1 mg. nicotinic acid	1.0	32
25 ” ” ” ” + 1 mg. nicotinic acid amide	0.7	21
75 ” ” ” ” + 70γ — 1 mg. nicotinic acid	2.2	35
20 ” Pyridine eluate of adsorbates II	1.2	7
20 ” ” ” ” ” ” + 70γ — 1 mg. nicotinic acid	0.9	32
20 ” ” ” ” ” ” + 1 mg. nicotinic acid amide	1.0	21
20 ” ” ” ” ” ” + 1-5 mg. adenylic acid or yeast nucleic acid	1.2	14

*A plateau period (± 4 g. in 10 days) ended depletion (average age of rats = 46 days) and preceded all assay periods.

†Lesions completely cured.

‡Symptoms partly cured.

§Lesions not cured.

cipitated much of the caramelized sugar but 50 mg. doses of the concentrated filtrate permitted a gain of only 0.8 gm. per day in the depleted animals. The alcoholic pyridine extract derived from the secondary adsorption products and previously assayed, was autoclaved for 8 hours at 120°C. The preparation in 20 mg. doses did not prevent the appearance of symptoms in some of the rats and allowed only a low rate of growth. These experiments supported previous observations with the autoclaved rice polishings concentrate that certain factors at different stages of their concentration could be altered by treatment at high temperatures.

The injection of growth hormone (anterior pituitary extract) in rats depleted of thiamin, riboflavin or the dermatitis factor failed to induce growth responses.

In view of the growth effects recently obtained with nicotinic acid and its amide,[5] these compounds were administered as supplements to animals receiving thiamin chloride and riboflavin, and to others which in addition were given preparations discussed above. The cereal grain included in the above ration[5] to provide suppletive amounts of the dermatitis factor makes comparisons difficult. The rice polishings concentrate and the extracts or eluates used in our experiments possibly contain nicotinic acid.†

Nicotinic acid (Eastman), 70γ, 140γ and 1 mg. daily, or nicotinic acid amide, 1 mg., did not stimulate growth in depleted animals on the sucrose diet with supplementary thiamin and flavin. These derivatives of pyridine had neither preventive nor curative action for typical symptoms of the florid dermatitis. Whereas the combination of the filtrate fraction with the alcoholic pyridine eluate produced an increased rate of growth, the addition of nicotinic acid or amide to this eluate did not, therefore, in rats, these compounds do not possess the complete properties of either the filtrate or the dermatitis factors.

A dose of 75 mg. of the rice polishings concentrate fed to depleted rats contained adequate quantities of the growth and dermatitis factors. A 25 mg. level under similar conditions resulted in a low growth rate which was not improved by the addition of nicotinic acid or amide. It appeared that this active component of the rice polishings concentrate was not identical with nicotinic acid or amide. In no case were delayed growth responses observed either when active fractions were fed to the rats or when they were maintained on the basal regime plus nicotinic acid or amide for four weeks.

Nicotinic acid amide, 1 mg., demonstrated no supplementary effect when administered together with 5 mg. of yeast adenylic or nucleic acids to animals receiving thiamin, riboflavin and the dermatitis factor (20 mg. pyridine eluate) over a 14-day period.

Summary. The extracts and eluates derived from rice polishings represented concentrated sources of the dermatitis factor as shown by curative properties and growth effects.

The filtrate from repeated adsorptions possessed properties which increase the growth-promoting action of the pyridine eluate.

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[5] Frost, D. V., and Elvehjem, C. A., *J. Biol. Chem.*, 1937, **121**, 255.

† Van Veen[6] identified adenine, uracil, cytosine, and nicotinic acid in the eluates from adsorption products of rice bran, and Funk[7] isolated crystalline nicotinic acid from purified extracts of rice polishings.

[6] Van Veen, A. F., *Mededeel. Dienst. Volks. Nederl.*, 1931, **20**, 80.

[7] Funk, C., *The Vitamines*, Baltimore, Williams & Wilkins, Trans. by Dubin, H. E., from the 2nd German ed., 1922, p. 171.

In this study nicotinic acid or amide were not equivalent to the filtrate or the dermatitis factors in the nutrition of rats. Nicotinic acid or amide had no supplementary effect when combined with concentrates from rice polishings and with yeast adenylic or nucleic acid.

9627 P

Effect of the Papilloma Virus (Shope) Upon the Tar Warts of Rabbits.

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When the papilloma virus is injected into the blood stream of rabbits previously tarred on the ears for 1½ to 3 months, growths rapidly arise on the tarred skin, often in great numbers. Some are squamous cell carcinomas,[1] usually multiple, and frequently metastasing.* We have studied the phenomenon in more than 70 rabbits, with 90 tarred controls. In none of the latter has a cancer developed.

Many of the growths that follow upon injection of the virus appear where no localized proliferation was previously visible, but others derive from pre-existing tar warts, which start growing rapidly, alter in aspect, and not infrequently manifest malignancy. After tarring is stopped, most ordinary tar warts disappear and the others become indolent, whereas the virus-stimulated warts keep on enlarging. Some are now carcinomatous but may undergo conversion into characteristic virus papillomas, as a scrutiny of several hundred specimens has shown; and others have become hybrids, neither ordinary tar tumors nor ordinary virus tumors but peculiar papillomas of malignant appearance, which change to squamous cell carcinomas almost at once. Not a few of the warts, however, though greatly stimulated by the virus (as proven by weekly records of their size), retain the morphology of the tar tumors.

To learn more, opportunity was provided for the virus to infect tar warts *in vitro*. The warty tissue was punched from the ears, a slice taken for section, and equal portions of the hashed remainder were steeped for some minutes in Tyrode, and in a Berkefeld filtrate of Tyrode containing potent virus, respectively; after which they were implanted at corresponding situations in the leg muscles and subcutaneous tissue of the hosts. Four or 5 wart materials from each rabbit were so treated, as was also some of the tarred skin devoid of warting. Tarring was kept up during a few later days because of its presumptive general influence to favor proliferation. The warts utilized proved to be the familiar tar papillomas and carcinoids,[2] so called because, though having the histology of carcinomas, and often ulcerating and growing through the ear, they ultimately disappear or revert to the benign, papillomatous form, even though tarring is continued.

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[1] Rous, Peyton, and Kidd, J. G., *Science*, 1936, **83**, 468.

* Using benzpyrene instead of tar, Lacassagne and Nyka have confirmed our findings (*Bull. de l'Assoc. Française pour l'Etude du Cancer*, 1937, **26**, 1).

One implanted animal died 20 days later and 9 were killed after 38 to 64 days. None of the 44 wart materials steeped in Tyrode had given rise to a growth, nor had the skin specimens done so. Tiny cysts lined with ordinary, stratified squamous epithelium resulted from 5 of the former and from 4 of the latter, the others undergoing resorption. Ferrero[3] has reported similar findings.

Very different was the outcome with the tissues steeped in virus. Then the bits of skin regularly gave rise to nodules of papillomatous tissue such as form on direct implantation of the Shope papilloma.[2] Similar nodules resulted from the implantation of 15 of the 24 tar papillomas, 4 yielded nothing, and 3 gave rise to small cysts like those just described. From one of the remaining 2 a malignant papilloma arose, markedly different from the original wart, and from the other a multicentric growth retaining the distinctive morphology of the latter. Nodules of Shope papillomatosis resulted from 8 of the 20 virus-steeped carcinoids, while 9 yielded nothing. In the remaining 3 instances, growths of carcinomatous aspect developed. The morphological characteristics of the original carcinoid had been retained in 2 of these cases, and the growths, though invasive, were small. The third was large, anaplastic and notably aggressive, to all appearances a malignant cancer, with but slight resemblance to the original carcinoid and none to those Shope papillomas that invade the muscle in which they are implanted.[4]

The warts from which the implantation growths of cancerous aspect derived had been large at biopsy in 4 of the 5 cases, and much of their tissue had been left *in situ*. After tarring was stopped one of them continued to enlarge slowly, but became a benign papilloma histologically, while the other 3 disappeared. The biggest to disappear was 2.3 cm. across and had extended through the ear when about one-tenth of it was taken for steeping. The bits steeped in Tyrode yielded no growth, whereas those exposed to virus gave rise to the large, malignant tumor just mentioned.

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[2] Borst, M., *Z. f. Krebsforsch.*, 1924, **21**, 344.

[3] Ferrero, V., *Arch. per le Sc. Med.*, 1926, **48**, 78.

[4] Rous, Peyton, and Beard, J. W., *J. Exp. Med.*, 1934, **60**, 701.

It is common knowledge that extraneous viruses[5] can flourish in tumors. Some induce no evident change, while others cause necrosis or the formation of inclusion bodies. The papilloma virus causes no inclusions, but it stimulates tar tumors to active growth, brings about morphological alterations in many of them, frequently acts as the determining factor in their survival, and makes some take on forthwith the character of carcinomas.

9628

Nutritional Myopathy of the Gizzard in Turkeys.

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This communication illustrates the fact that different species of animals may react in quite diverse fashion to the same abnormal diet. When growing chicks are given a simplified diet, lacking a still unidentified factor present in certain vegetable oils (Diet 108) extensive malacic lesions of the brain are produced.[1-4] The same diet fed to ducklings does not affect the brain, but leads to widespread degeneration of the skeletal muscles.[5] Pigeons appear to be refractory. We have recently found that the same diet given to turkey poults produces selective necrosis of the smooth muscle of the gizzard wall, unaccompanied by significant lesions in other organs or tissues. In this preliminary report, these alterations are described in detail. Further experiments are planned in the hope of obtaining more definite information as to the nutritional factors concerned.

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[5] Levaditi, C., and Nicolau, S., *Compt. rend. Soc. de Biol.*, 1922, **87**, 498; *Ann. Inst. Pasteur*, 1923, **37**, 443; Rivers, T. M., and Pearce, L., *J. Exp. Med.*, 1925, **42**, 523; Berry, G., and Syverton, G. P., *J. Bact.*, 1936, **32**, 356; Levaditi, C., and Haber, P., *Compt. rend. Acad. d. Sc.*, 1936, **202**, 2018.

[1] Pappenheimer, A. M., and Goettsch, M., *J. Exp. Med.*, 1931, **53**, 11.

[2] Pappenheimer, A. M., and Goettsch, M., *J. Exp. Med.*, 1931, **54**, 399.

[3] Pappenheimer, A. M., and Goettsch, M., Proc. Soc. Exp. Biol. and Med., 1934, **31**, 177.

[4] Goettsch, M., and Pappenheimer, A. M., *J. B. C.*, 1936, **114**, 673.

[5] Pappenheimer, A. M., and Goettsch, M., *J. Exp. Med.*, 1934, **57**, 35.

Thirty-one day-old Bronze and White Holland turkeys were kept in batteries for 6 days on a commercial turkey starter. They were then divided into two lots. Lot 1, (20 poults) was given Diet 108.[*] Lot 2 (10 poults) was maintained throughout the experiment on the original starting mash, and served as controls. On the experimental diet, the majority of the poults developed severe perosis within 18 days[†] whereas the controls remained free from this deformity. The growth rate of the experimental birds was inferior to that of the controls, but for the most part the turkeys were in good flesh, though of somewhat smaller size (Chart 1).

Most of the birds showed no definite signs of disease apart from the leg deformity and retardation of growth. Three, however, became weak and prostrated and acquired coarse tremors of the head—symptoms which could not be attributed to the perosis. At autopsy, the only significant lesion was found in the gizzard wall. The corneous lining was intact on gross and microscopic examination. There was nothing abnormal in the external conformation or tonus of the organ, but one could observe irregular greyish patches through the transparent serous covering. These were very conspicuous on section of the gizzard, appearing as rather circumscribed grey areas of firmer texture than the normal muscle (Fig. 1). Some of the patches had a pearly greyish luster, suggesting scar tissue. Others were more opaque. The lesions were not always sharply demarcated and conspicuous, but sometimes merged gradually into the healthy muscle, and were detectable only on close examination. The histological changes were in essence, hyaline necrosis of the smooth muscle fibers, with secondary inflammatory reaction, and later disappearance of fibers and replacement fibrosis. Individual fibers were swollen, lumpy and fragmented, staining more deeply with eosin than the normal elements (Fig. 2). The nuclei, instead of

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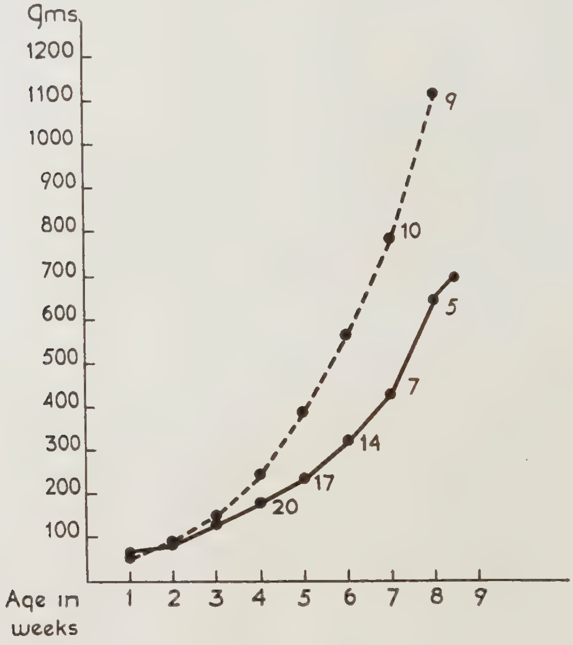
[*] Diet 108

Skimmed milk powder	15.0	Salt mixture (McCollum and Simmonds)	6.5
Casein, commercial	20.5	Paper pulp	10.0
Corn starch	20.0	Cod liver oil (Meade Johnson)	2.0
Lard	21.0		
Yeast (Bakers')	5.0		

[†] The severe perosis was probably due to the deficiency of Diet 108 in manganese (Wilgus, H. S., Norris, L. C., and Heuser, G. S., *J. Nutr.*, 1937, **14**, 155). Analysis showed merely a trace of manganese present.

Chart I
Growth on Experimental and Control Diets



Lot I - Diet 108
Lot II - Controls
Numerals indicate number of turkeys surviving

being pale and vesicular, became shrunken and pycnotic, later undergoing karryorrhexis, and eventually disappearing completely throughout the necrotic areas. In Masson trichrome preparations, the necrotic fibers could be sharply mapped out by virtue of their mauve color, which contrasted with the orange-red staining of the healthy fibers.

An acute inflammatory reaction was noted in and about many, but not all of the necrotic areas. The inflammatory cells consisted predominantly of pseudo-eosinophiles, with a varying proportion of large mononuclear cells. These were scattered amongst the necrotic fibers, and tended to accumulate also in the intermuscular septa and perivascular connective tissue (Fig. 3).

With the development of the lesions, the degenerated fibers gradually disappeared, and the supporting stroma of collagen and reticular fibers became condensed into scar-like tissue (Fig. 4).

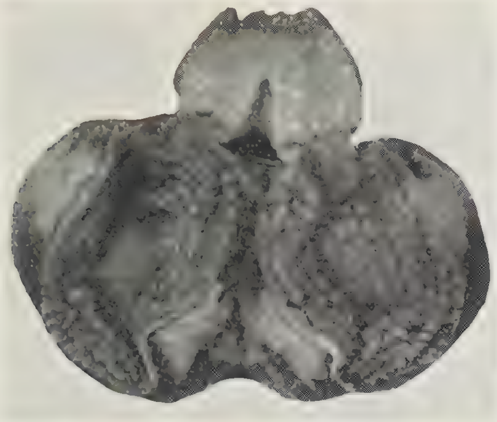


FIG. 1.
Gizzard, showing large patches of necrosis and fibrosis in muscle. The mucosa is intact.

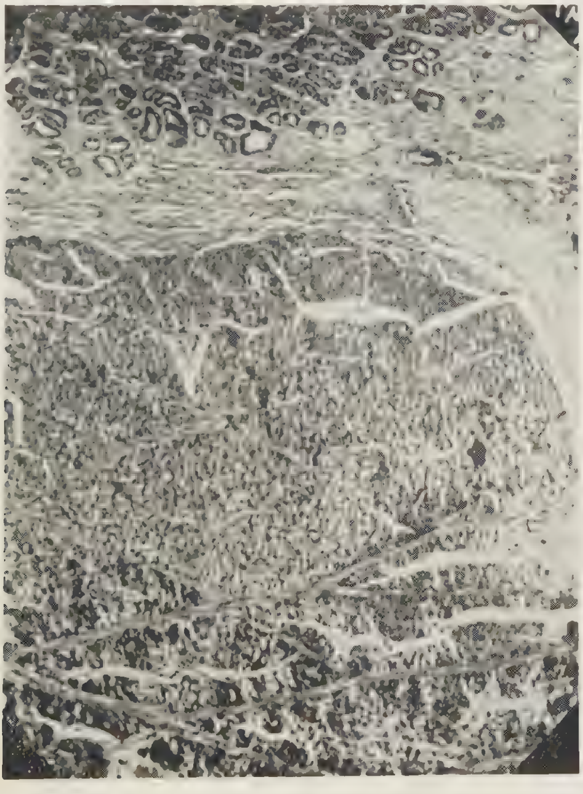


FIG. 2.
Section through lower portion of mucosa and portion of muscularis. The necrotic areas are more deeply stained. (Masson trichrome stain. × 110.)

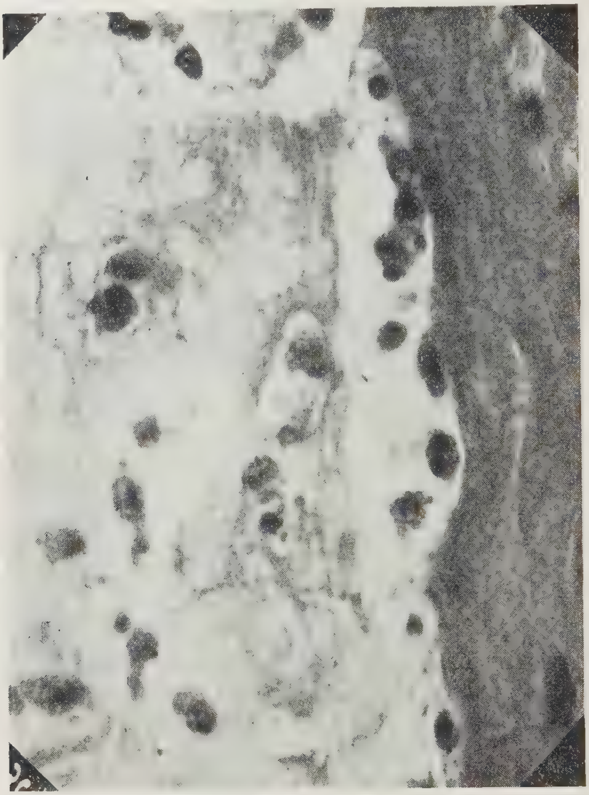


FIG. 3.
Necrosis of smooth muscle fibers. Inflammatory reaction, with pseudoeosinophile and mononuclear leucocytes. (H.E. × 1050.)

No changes were found in the blood vessels, and the ganglion cells and larger nerve trunks of the gizzard appeared normal with the non-specific staining methods used. Further study will be made of the neurites and nerve endings.

Apart from these lesions of the ventriculus, no constant or significant alterations were noted. The smooth muscle of the proventriculus, and of the intestine, of the aorta, and of the bronchi was not affected. The heart muscle also was unaltered, and the skeletal muscle showed no changes save occasional necrosis of individual fibers—possibly attributable to trauma associated with hock disease. The central nervous system, in contrast to the reaction in the brain of chicks maintained on this diet, showed no alterations. Focal necrosis of the pancreas was seen in one bird and of the liver in another. These were the only visceral lesions found in this series—

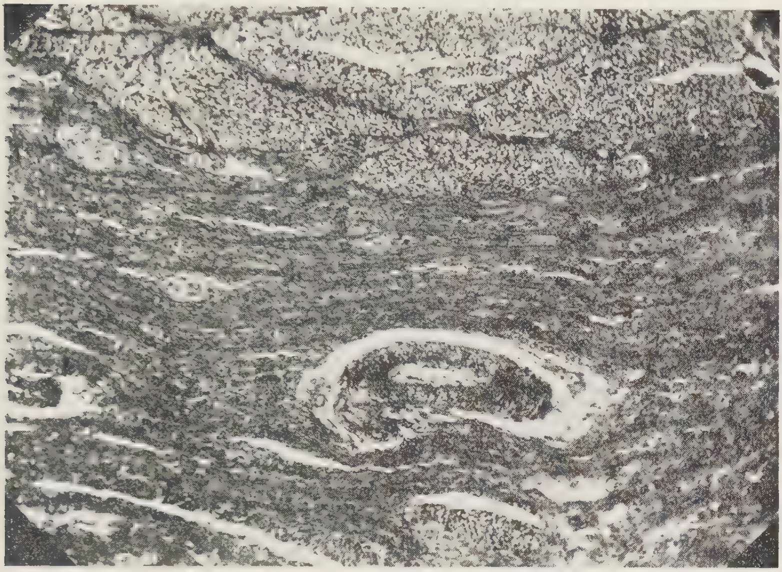


FIG. 4.
Large area of collapse fibrosis in gizzard wall. (Masson trichrome stain. × 110.)

except those described in the gizzard, and they were regarded as incidental.

Gross lesions like those described have never been observed in over 150 routine autopsies of turkeys. Three carefully studied controls on the commercial mash, showed no comparable gross or histological lesions in the gizzard, and the other viscera were normal. The other controls showed no evidence of illness, and are still under observation. Cultures were made from heart-blood and liver from 6 cases on liver infusion agar, and meat extract agar. No pathogenic organisms were recovered. No bacteria were seen in the stained sections.

An analysis of the data given in Table I shows that gross lesions of the gizzard wall were present in 11 of 14 turkeys which survived for 30 days or longer upon the experimental diet, and 2 additional positive cases were detected on microscopic examination. The incidence was thus almost 93%.

We have found in the literature no reference to similar lesions of the gizzard. Inflammatory foci of the gizzard wall which grossly resemble those here described, do occur in pullorum disease of chicks,[6] the predominant cell type being mononuclear. In a case

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[6] Van Heelsbergen, T., *Hdbch. d. Geflügelkrankheiten, etc.*, F. Enke, Stuttgart, 1929, p. 116.

TABLE I.

			Gizzard Lesions			
				Microscopic		
No.	Days on Diet 108	Final Weight	Gross	Necrosis	Inflammation	Fibrosis
1	K 30	308+	+++	++	+	—
2	K 39	304	++++	++	+	—
3	D 33	420+	+	++	+++	—
4	D 18		Found dead and decomposed—no tissues			
5	K 50	528	—	+	—	—
6	K 21	190	Not examined			
7	K 50	571	±	+	—	+
8	K 33	268+	Not examined			
9	K 37	352+	+++	+++	+	+++
10	K 50	961	+	++	—	+
11	K 50	660	+++	+	+++	—
12	K 50	658	++	+	—	+++
13	K 39	330	++	+	—	+++
14	K 36	183+	+++	+++	+	—
15	D 24	239	Not examined			
16	K 23	190+	” ”			
17	K 37	272+	+++	+	+	—
18	K 32	305	±	—	—	—
19	K 36	275	+	+	—	+
20	D 18	108	Not examined			

recently seen bacilli were easily demonstrated in the lesions.

Under the title of lymphoblastoma, Adamstone[7] has recently described lesions of the gizzard, liver and other organs observed in chicks reared on a diet in which vitamin E was destroyed by ferric chloride treatment. It is not possible to decide from the photographs or description whether these lesions resemble those found in our turkeys. Chicks on Diet 108, which is lacking in vitamin E, have not, in our experience, shown similar lesions of the gizzard wall, although erosions of the corneous lining are frequently seen. It would seem then, that we are dealing with a previously unrecognized nutritional disorder of turkeys characterized by a specific lesion of the gizzard muscle.

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[7] Adamstone, F. B., *Am. J. Cancer*, 1936, **28**, 540.

9629

Blood Sugar Response to Intraperitoneal Epinephrine Injections in Normal and Hypophysectomized Dogs.

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Aschner[1] showed that epinephrine subcutaneously administered produces less glycosuria in the hypophysectomized dog than in the normal. Other investigators[2, 3, 4] have since shown that epinephrine so administered produces less elevation of the blood sugar in hypophysectomized toads, rabbits and dogs than in the normal animals. Until the recent report of Russell and Cori[5] the impression therefore prevailed that the glycogenolytic action of epinephrine is diminished in hypophysectomized animals. These investigators demonstrated, however, that in the rat intravenously administered epinephrine produces an effect on blood sugar similar to that obtained in normal controls. In the course of certain investigations on the carbohydrate metabolism of hypophysectomized dogs observations were made by us on the effect of intraperitoneal injections of epinephrine on blood sugar which are herein reported.

Nine normal and 9 completely hypophysectomized dogs were injected intraperitoneally. In 3 animals observations were made under identical conditions before and after hypophysectomy. The dosage used was 0.75 cc. (1-1000) in 10 cc. of normal saline per 6 kg. of body weight. Coincidental studies on insulin sensitivity revealed that the hypophysectomized animals were 2 to 4 times as sensitive as the normals. Autopsy later established the completeness of the hypophysectomy. Some of the dogs studied were on a balanced diet of carbohydrate, fat and protein, others were on a high fat, low carbohydrate diet and a third group was on a low fat, high carbohydrate diet. The alterations in diet made no appreciable difference in the effectiveness of epinephrine as a glycogenolytic factor. The effect on blood sugar was determined by analyses made at frequent intervals, up to 6 hours, using the Shaffer and Somogyi sugar method.[6]

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[1] Aschner, B., *Pfluger's Arch.*, 1912, **146**, 1.

[2] Houssay, B. A., and Benedetto, E., *Compt. rend. Soc. Biol.*, 1932, **111**, 472.

[3] Bachman, C., and Toby, G., *J. Physiol.*, 1936, **87**, 1.

[4] Chaikoff, I. L., Reickert, F. L., Read, L. S., and Mathes, F. E., *Am. J. Physiol.*, 1935, **113**, 306.

[5] Russell, J. A., and Cori, G. T., *Am. J. Physiol.*, 1937, **119**, 167.

[6] Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 695.

TABLE I.

Blood Sugar Response to Intraperitoneal Epinephrine Injection in Normal Dogs. Figures represent mg. of glucose per 100 cc. blood.

Dog No.	Date 1936	Time in min. after administration epinephrine 0	30	60	90	120	180	240	300	360
319	6/23	84	104	120	—	130	129	105	94	96
06	10/7	68	97	116	113	102	90	92	108	118
A.6	10/7	60	73	88	101	113	112	104	102	99
B.38	6/23	86	127	147	—	158	154	105	95	108
B.51	6/23	70	92	102	—	112	128	116	92	89
A.6	10/7	60	73	88	101	113	112	104	102	99
B.45	10/14	68	86	126	139	130	88	86	83	84
1	10/14	74	88	108	113	114	99	93	92	88
78	12/18	83	107	104	118	91	96	90	89	88

Dosage = 0.75 cc. 1:1000 epinephrine in 10 cc. n/saline per 6 kg. of body wt.

TABLE II.

Blood Sugar Response to Intraperitoneal Epinephrine Injection in Hypophysectomized Dogs. Figures represent mg. of glucose per 100 cc. blood.

Dog No.	Date 1936	Time in min. after administration epinephrine 0	30	60	90	120	180	240	300	360	Diet
319	7/8	79	100	117	—	108	88	89	81	93	Normal
06	11/16	57	82	96	114	114	83	71	84	86	,,
A.6	11/16	59	63	110	128	121	82	64	69	69	,,
112	6/26	60	80	113	—	160	128	93	69	62	,,
A.4	6/3	75	89	127	151	116	81	83	88	—	,,
A.7	12/4	76	97	128	143	133	94	58	69	77	,,
113	5/9	77	102	110	128	133	126	—	—	—	High Fat
A.2	6/3	79	93	117	119	110	88	88	85	—	,, CHO
A.2	6/26	62	83	101	—	119	93	60	60	69	Normal
A.2	10/4	58	68	98	107	97	81	53	50	59	,,
A.1	6/26	62	89	91	—	96	89	77	74	78	,,
A.5	6/26	64	80	98	—	97	83	75	78	82	,,

Dosage = 0.75 cc. 1:1000 epinephrine in 10 cc. n/saline per 6 kg. of body wt.

Analysis of the results outlined in Tables I and II shows that epinephrine intraperitoneally administered is in general as effective in raising the blood sugar in hypophysectomized dogs as in the normal. Glycogen determinations on animals A.1 and A.2, Table II, carried out in the post-absorptive state, showed 5.4% and 6.1% glycogen content for the liver and 0.75% and 1.06% for the muscle of the 2 animals. The method of analysis used was that of Good, Kramer and Somogyi.[7] It is apparent that hypophysectomized dogs, in a state of good nutrition, have an adequate liver and muscle glycogen supply to serve as substrate in the glycogenolytic process.

The reason for the lessened effect on blood sugar from subcutaneously administered epinephrine has not been definitely established. Russell and Cori suggested that it probably was due to a difference in the rate of absorption. Evidence in keeping with this explanation has been provided by one of us[8] in studies on the sensitivity of smooth musculature to exogenous epinephrine in cats as altered by hypophysectomy. It was found that the smooth musculature of such animals shows a definitely hyperdynamic contractibility (30-40%) on contact with epinephrine. This would result in an accentuated contraction of the blood vessels at the site of subcutaneous injection with a corresponding slowing of the rate of absorption. Such a factor would not be active on intravenous or on intraperitoneal injection where absorption would occur largely through the lymphatics.

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[7] Good, C. H., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 485.

9630

Photoelectric Plethysmography of the Fingers and Toes in Man.

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The detection of changes in the blood content of tissues with the aid of the photoelectric cell has been reported by several observers.[1] This paper reports the development of photoelectric plethysmographs which lend themselves to clinical use in dynamic analysis of the peripheral circulation and which may be "plugged in" in place of the patient's lead on the electrocardiograph. A summary of some observations thus recorded on the finger is reported elsewhere.[2]

A beam of light is directed from an ordinary automobile headlight bulb (direct current) on the finger or toe placed above a shielded photoelectric cell of the photo-emissive type, purchased in the radio trade, (cell current generated is approximately equal to 5×10^{-10} ampere—this may be increased by impressing 45-90 volts across the cell). The photoelectric oscillations with variations in the blood content of the digit are recorded by a string galvanometer or suitable oscillograph after amplification.

Adaptation of the principle of the method to the finger is shown in Fig. 1. A comfortable saddle or sling for the arm is necessary to

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[8] Heinbecker, P., *Am. J. Physiol.*, 1937, **120**, 401.

[1] Bonsmann, M. R., *Arch. f. exp. Path. u. Pharmakol.*, 1934, **176**, 460; Matthes, K., *Ibid.*, 1935, **179**, 698; Hanzlik, P. J., DeEds, F., and Terada, B., *J. Pharm. and Exp. Therap.*, 1936, **56**, 194; Molitor, H., and Kniazuk, M., *Ibid.*, 1936, **56**, 1.

[2] Hertzman, A. B., and Spealman, C. R., *Am. J. Physiol.*, 1937, **119**, 334.

FIG.1

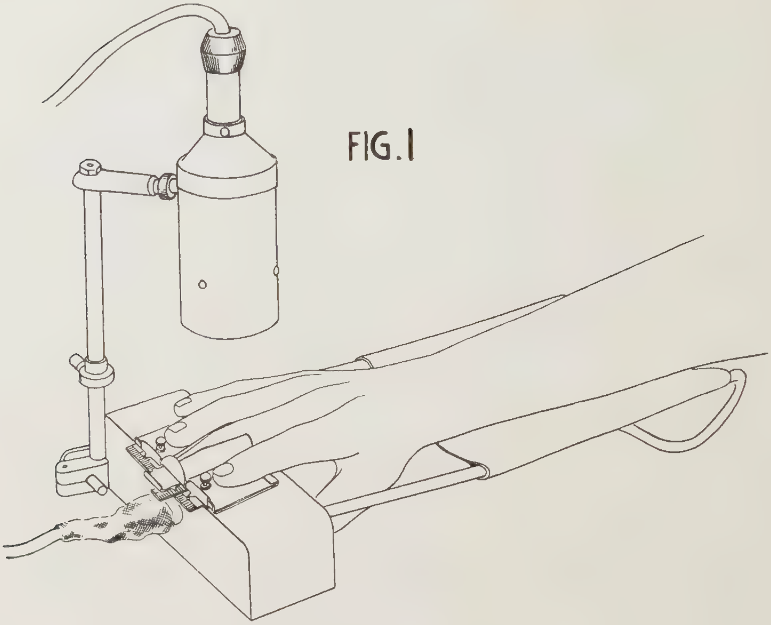

Fig. 1.
Finger photoelectric plethysmograph.

FIG.2

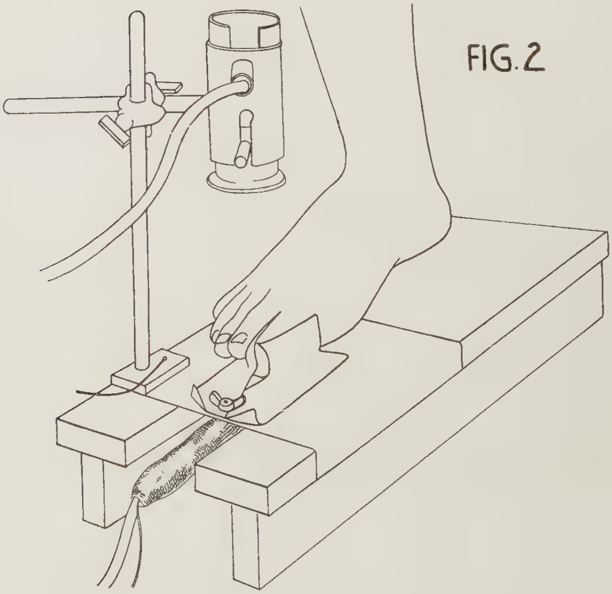

Fig. 2.
Toe photoelectric plethysmograph.

secure the desired muscular relaxation which greatly affects finger volume. Movements of the arm must not be transmitted to the finger. A pillow may be substituted for the saddle and the method used on the subject in bed. The light and photoelectric cell may be placed in any plane desired.

In the arrangement for the toe (Fig. 2), a heel plate (not illustrated) prevents movement of the foot. The method may be used on the subject when standing, sitting, or reclining. If the light, toe mask and photoelectric cell are mounted on a sliding base, fitting the plethysmograph to feet varying in length and to individual toes of the same foot is quickly and conveniently accomplished. It is convenient to have a battery of toe masks of various sizes and shapes to take care of the great variations in toes.

The finger volume pulse, thus recorded, is illustrated in a previous paper[3] in comparison with the volume pulse of the nasal septum, similarly recorded. The amplitude of the digit volume pulse, the form of the wave, the position and size of the dicrotic wave, alter with circulatory dynamics the study of which is thus conveniently and profitably aided by the photoelectric technique (Fig. 3).

The photoelectric technique may be readily used as an electric

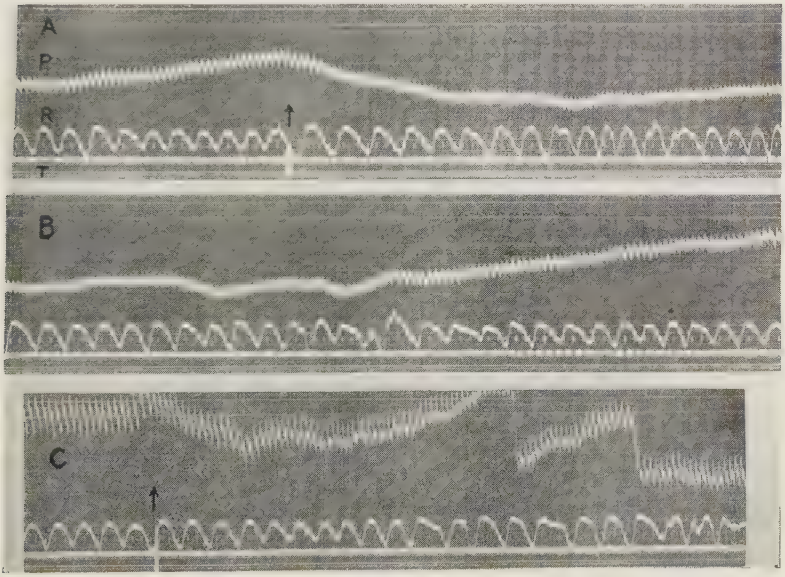

FIG. 3.
P—finger photoelectric plethysmogram. R—respiration. Time—1 second. Amyl nitrite at arrow in records A and C. Record B continuation of A.

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[3] Hertzman, A. B., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 290.

plethysmograph to study simultaneously the effects of various procedures on digit blood volume as well as on the form and amplitude of the digit volume pulse. Illustration is provided in Fig. 3 showing the effects of amyl nitrite on finger blood volume and finger volume pulse.

Validity of the Method. This has been examined through the execution of experiments along two general lines: (1) comparison of the photoelectrically recorded changes in finger blood volume with the changes in finger volume simultaneously recorded (on the same finger) by the transmission type of plethysmograph. The sensitivity of the photoelectric recorder in the several trials was measured in arbitrary units by the insertion of a suitable absorption filter between light and finger. (Although the *in vitro* whole blood and hemoglobin equivalents of the filter were readily determined, it was found that these values were irrelevant for the purpose.) The blood equivalents of these arbitrary units were calculated from the data supplied by the transmission plethysmograph. The photoelectric recorded pulse volume could then be calculated and compared with the pulse volume recorded by the transmission plethysmograph and with normal pulse volumes recorded in the literature. It is apparent from Table I that the photoelectric recorder is semiquantitative. The indeterminate character of the finger geometry involved in the transmission of light to the photoelectric cell precludes expectation of precise quantitation and prohibits the transfer of the plethysmographically determined blood equivalent of the filter from one individual to another. One is, therefore, compelled either to assign an arbitrary value to the filter used in determining recorder sensitivity or, when blood equivalents are desired, to actually determine these by means of transmission plethysmographs, in each individual digit studied. The former procedure is apparently compatible with quantitatively equivalent studies and readily permits a study of peripheral circulatory mechanics, so that actual blood equivalents become desirable only in rare instances. This is important not only for simplicity and convenience in the use of a photoelectric plethysmograph but also for application to regions such as the nasal mucosa which cannot be satisfactorily studied with transmission plethysmographs. Unfortunately, in such cases, the question as to what is the tissue blood equivalent of the filter becomes extremely complex. Space limitations preclude the consideration of the effects of variations in red blood cell form and number, of changes in the oxygen saturation of hemoglobin, of the axial stream effect in small vessels. These factors bear on the quantitation of

TABLE I.
Summary of Calibration Data.

Exper.	Increase in Finger Volume (Plethysmograph), cc.	String Deflection mm.	String Deflection to Red Filter, mm.	Blood Vol. Equivalent to String Deflection, cc./mm.	Blood Equivalent to Red Filter cc.	Pulse Vol. Plethysmograph cc.	Pulse Vol.—Photoelectric (Calculated) cc.
$(1)_1$	0.21	28	24	.0075	.18	.012	.015
$(1)_2$	0.29	40	24	.0072	.173	.0102	.0144
$(2)_1$	0.30	19	9	.0158	.142	.0126	.0125
$(2)_2$	0.48	24	7.5	.02	.15	.0133	.016
$(2)_3$	0.31	20	13.5	.0155	.209	.0077	.0108
$(2)_4$	0.28	27.5	31	.0102	.316	.0140	.0275

the photoelectric recorder but apparently do not significantly influence the qualitative value of its records.

(2) The essential validity of the photoelectric method is further supported by comparison of the photoelectric records with instances in which independent confirmation by other methods is available in the literature. Thus: 1. A pulse volume (photoelectric) of .01-.02 cc. compares favorably with the values of Johnson[4] and of Goetz[5] obtained with optically recording plethysmographs. 2. Quiet breathing has only negligible effects on finger volume and on the volume pulse. Compare Goetz.[5] 3. A marked vasoconstriction in the finger is observed at the time of a deep respiration in a quietly resting individual. One is reminded of the demonstration[6] that the phenomenon is a vasoconstrictor reflex not obtained in denervated and sympathectomized limbs. 4. The changes in amplitude and form of the volume pulse recorded in the valsalva experiment were identical with those obtained by Goetz[2] using transmission plethysmograph. 5. Amyl nitrite (Fig. 3) is followed by decreasing finger blood volume, decreased pulse amplitude and a collapsing type of wave indicating arterial relaxation in the finger with drainage of blood to other areas. Johnson[7] obtained similar results. Additional instances have simply illustrative value in establishing confidence in the validity of the records taken with the photoelectric plethysmograph.

Summary. Photoelectric plethysmographs for the fingers and toes are described which use electrocardiographs for the recording and which have definite advantages in routine clinical observations on the circulation. The validity of the technique is established (1) by comparison of the photoelectric records with simultaneous records obtained with transmission plethysmographs, (2) by comparison of the photoelectric records in instances of circulatory disturbances with independent directional confirmation by other methods in the literature.

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[4] Johnson, C. A., *Surg. Gynec. and Obst.*, 1932, **55**, 731.

[5] Goetz, R. H., *Pfluger's Arch. f. d. ges. Physiol.*, 1935, **235**, 271.

[6] Bolton, B., Carmichael, A. E., and Stürup, G., *J. Physiol.*, 1936, **86**, 83.

[7] Johnson, C. A., *J. Lab. and Clin. Med.*, 1932, **17**, 59.

9631

Effect of Hypotonic and Hypertonic Ringer on Isotonic and Isometric Contraction of Muscle.

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It was shown by Cooke[1] and Meek[2] for striated muscle and by Carlson[3] for heart muscle that the height of contraction produced by a constant stimulus increased when the muscle was put in hypotonic saline solution. Later it was shown by Lapique[4] that the irritability of the muscle was increased (decreased chronaxia) by treatment with hypotonic solutions. All of these observations conform with the idea that active (young) tissues contain more water than inactive (old) ones, that stimulation is due to the heaping up of ions at some membrane and the more water there is the easier it would be for ions to move. However, the fact that an isotonic lever goes higher does not prove that increased tension was developed in the muscle and the muscle therefore able to do more work. There is an inverse ratio between speed and work in muscle contraction and if the tension is due to some kind of surface action one would not be surprised if the tension developed might be independent of the water in the cell. On testing the effect of hypotonic Ringer with an isometric lever we find, as described below, the tension developed does not increase but rather tends to decrease with the uptake of water.

The experiments were performed by suspending a frog's gastrocnemius muscle in a special glass chamber mounted on the stand of an ordinary moist chamber. The glass chamber afforded a means for surrounding the muscle with Ringer's solution when determinations were not actually being made. In all cases the right and left gastrocnemius muscles of the same frog were used; the right was surrounded with isotonic Ringer's solution and served as a control to the left gastrocnemius, which was considered the experimental muscle and was surrounded with 25% hypotonic solution. Single induced break shocks were used in which the strength of the current in the primary was kept constant. Contractions were recorded on a stationary drum at 15-minute intervals over a period of 3 hours. The first contraction in each case was taken immediately after the muscle was mounted in the chamber to serve as a control, after which the solutions were added to the chamber.

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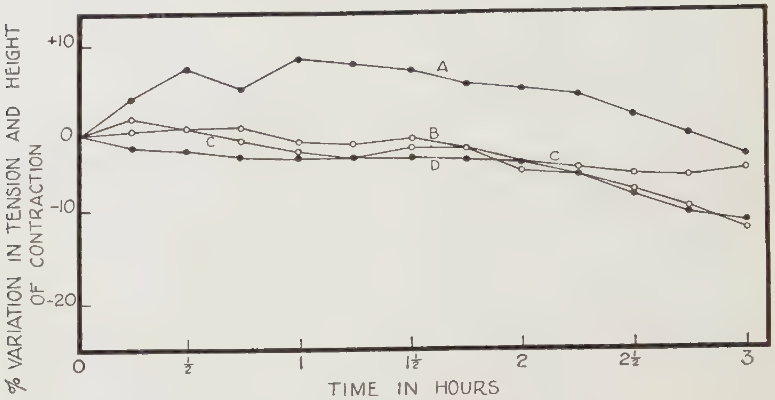
[1] Cooke, E., *J. of Physiol.*, 1898, **23**, 137.

[2] Meek, W. J., *Am. J. Physiol.*, 1906, **17**, 8.

[3] Carlson, A. J., *Am. J. Physiol.*, 1906, **15**, 357.

[4] Lapique, M., *Compt. rend. Soc. de biol.*, 1920, **83**, 1033.



As the plate shows, when the isotonic lever was used, the height of contractions of the muscle in hypotonic Ringer's solution was above that of the control and tended to remain so for at least an hour and a half. Curve A represents the percentage variation in height of contraction of the muscle in hypotonic, and Curve B the control muscle in isotonic Ringer's solution. The results obtained with the isometric lever under the same conditions, however, show no appreciable difference between the control and experimental muscles. (Curves C and D respectively.) The lever used was somewhat similar to that devised by C. F. Palmer Co. and figured in Starling's text. Each curve represents an average of 6 experiments.

It follows, then, that although the muscle definitely took up water and no doubt was more irritable than its control, it was not able to exert any more muscular tension.

Experiments were also carried out to test the effects of 25% hypertonic Ringer's solutions on muscle tension. It was noticed that with both the isotonic and isometric levers the muscles showed a definite decrease in muscular tension compared with the control.

Summary. When muscle is treated with hypotonic Ringer an increased height of contraction is obtained from a constant stimulus with an isotonic lever but if an isometric lever is used it shows there is no increased tension developed by the muscle. Hypertonic solutions lead to decreased height of contraction and decreased tension.

9632 P

Glucose Infusion Through the External Pudic Artery.*

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In previous work the amount of lactose in the milk of cows was reduced following partial depletion of the blood glucose through the administration of insulin.[1] This led to attempts to study the effect of increased blood sugar levels upon the amount of lactose in the milk. All attempts at increasing the blood sugar for any period of time failed when glucose was injected intravenously.[2] Intramammary duct injections of glucose solutions were also used but resorption of glucose by this method also failed to increase the blood sugar.[3]

To study the effect of increased blood sugar upon the quantity of lactose formed in the milk it became apparent that the additions of glucose must be made to the arterial blood and preferably immediately before entering the mammary gland. If glucose is added to the arterial blood of the external pudic artery, the arterial blood passing through the mammary gland will be augmented to the extent of the addition. Accordingly, the technique described by Boyd and Petersen[4] was employed to secure puncture of the external pudic artery and isotonic solutions of glucose were infused over periods up to 6 hours in duration.

The infusion was effected by elevating the aspirator to cause flow by gravity. During the infusion the experimental subject was milked at frequent intervals and the urine was sampled for analysis. As the infusion was performed on the right side of the udder the milk from the left side served as a check as well as the samples before the experiment.

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* Published with the approval of the Director as Paper No. 1380, Scientific Journal Series, Minnesota Experiment Station.

[1] Petersen, W. E., Hewitt, E. A., Boyd, W. L., and Brown, W. R., *J. Am. Vet. Med. Assn.*, 1931, **79**, 217; Brown, W. R., Petersen, W. E., and Gortner, R. A., *J. Dairy Science*, 1936, **19**, 147.

[2] Brown, W. R., Petersen, W. E., and Gortner, R. A., *J. Dairy Science*, 1936, **19**, 177.

[3] Brown, W. R., Petersen, W. E., and Gortner, R. A., *J. Dairy Science*, 1936, **19**, 243.

[4] Boyd, W. L., and Petersen, W. E., to be published.

The lactose contents of the milks for both halves of the udder are given for: (a) the average of 3 days' preliminary milkings, (b) 3 milkings during the experiment, and (c) the next 2 milkings following the experiment.

TABLE I.
Influence of Infusion upon Lactose Content of Milk.

Exp.	Side of Udder	Preliminary %	Experimental Milks 1st %	2nd %	3rd %	Two Milkings following Experiment Evening %	Next morning %
1	Right	4.2	4.4	4.2	4.5	5.7	5.2
	Left	4.3	4.7	4.1	5.0	5.8	5.6
2	Right	4.6	4.7	4.6	4.1	4.6	4.7
	Left	4.7	4.7	4.7	4.5	4.6	4.6
3	Right	4.7	5.4	4.8	5.2	4.9	5.0
	Left	4.8	5.6	4.7	4.7	4.8	5.1
4	Right	4.9	5.0	4.8	4.9	4.7	5.0
	Left	4.8	4.8	4.9	4.9	4.8	5.1

TABLE II.
Duration of the Experiments and the Quantities of Isotonic Glucose Solution Infused.

Experiment No.	Duration Hr.	Amount infused Liters
1	4	15
2	6	27
3	6	24
4	5	20

It is to be noted that there is from a slight to a significant increase of lactose in the milks following the experiment and varied effects upon the lactose during the infusion. As the effects are the same for both halves of the udder the results cannot be attributed to the increased glucose content of the blood.

In a few cases the blood sugar was determined upon the venous blood secured from mammary vein punctures close to the udder. The total sugar content varied from 206 to 361 mg. %. All experiments were accompanied with marked diuresis. The total reducing sugar content of the urine, according to the Tauber and Kleiner method,[5] reached a high in the middle of experiment 2. At this point there were 1.5% total sugar and 0.308% lactose. The urine before the experiment averaged .110 total reducing sugar and .064% monose.

It is apparent from the data presented that the lactose content of milk is not increased by increasing the glucose content of the arterial blood. Milks following the perfusion experiments have increased lactose contents that must be explained on some other basis than the effect of increased glucose content of the blood.

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[5] Tauber, H., and Kleiner, I. S., *J. Biol. Chem.*, 1933, **99**, 249.

9633 P

Limits of the Optically Active Cortex of the Rabbit.*

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The approximate coincidence of the optically excitable area with the area striata as defined histologically may be inferred from the work of Kornmuller[1] on the Berger rhythm, and of Bartley and Bishop[2] on responses to stimulation of the optic nerve. We have here examined the limits of optic cortex histologically and physiologically in the same animals. The physiological technique has been described (Bartley and Bishop[2]).

Ten animals were used. In 5 the indifferent electrode was fastened to bone adjoining the exposed cortex; in 5, it was plunged through the cortex to the subjacent white matter. In the animals of both series the local recording electrode was moved across the margins of the optic area, recording consecutively from series of points. Identifying stabs served to locate recorded points in 20 micron frontal sections cut serially from the cortex studied. The serial sections from each brain were used to reconstruct the histological boundaries of the area striata at 10 diameters (Fig. 1A). Upon these reconstructions were entered the physiological data.

The *lateral* border of the area striata, determined by the histological criteria of Rose,[3] coincided closely with the functionally defined limit of the optically active area. In our histological preparations a transition zone of 1 to 2 mm. width occurred between the area striata and the neighboring area occipetalis which we could not assign exclusively to either field. The stabs which identified recorded

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* Aided by a grant from the Rockefeller Foundation to George H. Bishop for research in neurophysiology.

[1] Kornmuller, A. E., *Biol. Rev.*, 1935, **10**, 383.

[2] Bartley, S. H., and Bishop, G. H., *Am. J. Physiol.*, 1935, **10**, 149.

[3] Rose, M., *J. Psych. u. Neur.*, 1931, **43**, 353.

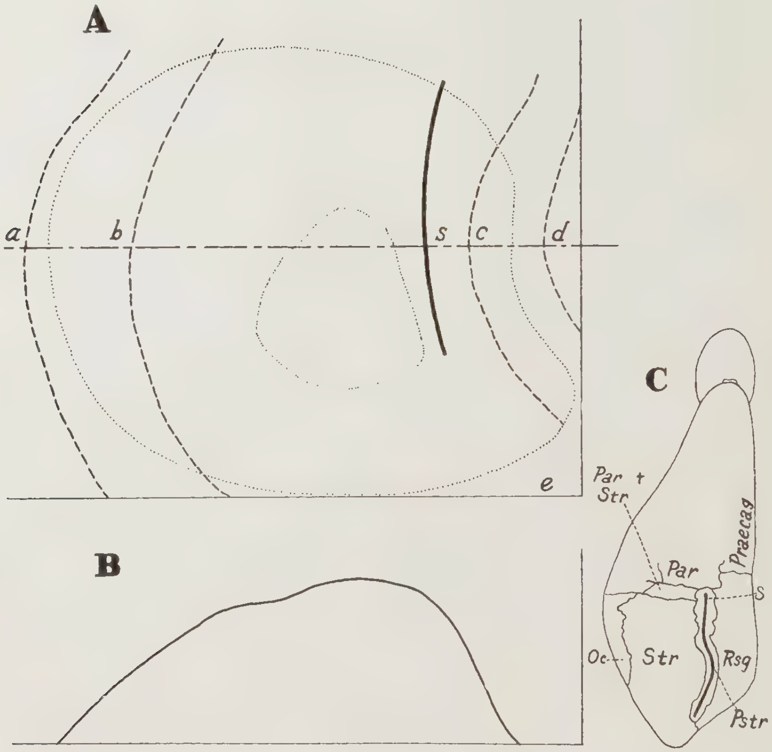

Fig. 1.

A. Map of the histological and functional boundaries considered significant in the interpretation of these data. Broken lines *a* and *b* indicate the limits of the transition zone between the occipetal and striate areas, broken lines *c* and *d* of the transition zone between the parastriate and retrosplenial areas. *S,* heavy line, gives the position and extent of the parasagittal fissure. Line *e* passes through the posterior pole of the cortex; line *m,* perpendicular to *e,* is the midline. Outer dotted line shows the limit of the optically excitable area, *i. e.,* the locus of points at which a single electric shock applied to the contralateral optic nerve induces a just visible response. Inner dotted circle indicates the region of the optically active cortex where the response is highest.

B. Profile illustrating the gradient in amplitude of potential response along the line *a-d* across the optically active cortex. This figure represents the average results of all experiments.

C. Diagram of the dorsal surface of the left cerebral hemisphere of the rabbit modified from Rose's corresponding map of the right cerebral hemisphere. The original Rose diagram has been simplified to suit the present purpose. *Str,* area striata; *Par + Str,* transition field between the areas striata and parietalis; *Oc,* area occipetalis; *Pstr,* parastriate area; *Rsg,* retrosplenial area. Heavy black line, *S,* has been inserted to indicate the extent of the parasagittal fissure referred to in the text. It corresponds to the *S* of 1 A.

points of minimal response on the lateral margin fell within the histological transition zone.

The *medial* border of the histologically defined area was significantly overlapped by the optically active area in each of the 10 animals used in the experiments. Records taken from serial points on frontal planes between the anatomical center of the area striata and the midline (Fig. 1B) demonstrated that the medial margin of the optically active area fell within the transition zone, about 2 mm. wide, between the areas parastriata and retrosplenialis (Figs. 1A and 1C). The area parastriata in the rabbit, which underlies a shallow parasagittal fissure, is, therefore, a part of the optic projection area; and from this and other evidence we conclude that it represents no more than a fissural adaptation of the histological pattern of the area striata.

The *anterior* histological border coincides approximately with the limit of the optically active cortex in this direction.

The *posterior* margin determined histologically or physiologically falls at the posterior pole of the hemisphere. Due to the rapid curving of the cortex at this point and the consequent disarrangement of the layer pattern in frontal sections, the correlation of histological and electrophysiological data was less reliable than on the other borders.

With the complication noted at the medial border, the coincidence between the previously defined architectonic area striata and the field of optically induced cortical response is satisfactory. This appears to justify utilizing the electro-physiological method in defining the boundaries of other projection areas from which induced responses can be recorded following stimulation of the appropriate sensory nerves.

9634

Co-Carboxylase (Vitamin B_1-Pyrophosphate) Content of Plants.

Henry Tauber.

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Lohmann and Schuster[1, 2] have shown that co-carboxylase is identical with the pyrophosphoric ester of vitamin B_1. I have demonstrated recently[3, 4] that synthetic vitamin B_1 is readily phosphorylated by the duodenal mucosa and by extensively washed dry yeast

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[1] Lohmann, K., and Schuster, P., *Naturwiss,* 1937, **25**, 26.

[2] Lohmann, K., *Angew. Chem.,* 1937, **50**, 221.

[3] Tauber, H., *Science,* 1937, **86**, 180.

[4] Tauber, H., *Enzymologia,* in press.

which had been freed of co-carboxylase. The enzymic synthesis of vitamin B_1-pyrophosphate occurs best at a slightly acid pH. Euler and Vestin,[5] and Kinnersley and Peters[6] independently and at the same time when I published my experiments have also shown that yeast converts vitamin B_1 to co-carboxylase.

The enzyme carboxylase has an important part in carbohydrate metabolism. It is widely distributed in nature and it is accompanied by its co-enzyme. It occurred to me that a quantitative study of a series of plants for co-carboxylase and comparing these findings with the vitamin B_1 content of the plant material may be of interest.

The well ground plant tissues were extracted by boiling for 5 minutes with 3 to 5 times their weight of pH 6.239 phosphate (Sörensen). In 2 cc. of the clear filtrates co-carboxylase was determined according to Lohmann and Schuster.[1] In Column 1 of Table I, figures obtained for co-carboxylase are shown and in Column 2, figures for vitamin B_1 content of these plant materials as obtained by biological assay are given. By comparing these figures with that which I have obtained for the enzymatically synthesized co-carboxylase (not completely phosphorylated) it may be seen that only a

TABLE I.
Analysis of Co-carboxylase per gm. of Plant Material.

	Cmm. CO_2	Gamma of vitamin B_1*
Aspergillus Niger, mycelium pressed free of water	65	—
Cabbage, bleached	80	125-130
Onions	120	50-200
Orange, juice	80	150
,, peel	125	—
,, seeds	250	—
Pecans	170	300
Pepper, green	86	25-50
Spinach	10	125-250
Squash, summer, soft, seeds and all	107	875
Turnip, greens	93	125
Yeast, dry, bakers'	1600	650-2250
Co-carboxylase, enzymatically synthesized, equivalent to 5 gamma vitamin B_1	56	5

The Warburg-Barcroft apparatus was employed. Temperature 35°. Time 60 minutes. Volume of Warburg vessels 16 cc. In side arms 0.5 cc. of pyruvate solution (containing 5 mg. of pyruvic acid and 0.1 mg. of magnesium as $MgCl_2$) of pH 6.2. In the main room of the vessels 2 cc. of plant extract and 1 cc. of dry washed yeast.[4] The extensively washed dry yeast employed as carboxylase was practically free of co-carboxylase.[4]

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[5] Euler, H. v., and Vestin, V., *Naturwiss,* 1937, **25**, 416.

[6] Kinnersley, H. W., and Peters, R. A., *J. Chem. Ind.,* 1937, **56**, 447.

* From Hawk and Bergeim.[7]

[7] Hawk, P. B., and Bergeim, O., *Practical Physiological Chemistry,* 11th edition, Philadelphia, 1937, p. 837.

small part of the total vitamin B_1 is present in these plants in the form of its pyrophosphoric ester. It should be noted, however, that there is no direct proportionality between CO_2 formation and the amount of co-carboxylase added, especially when too large amounts of the co-enzyme are to be estimated. For this reason in the case of dry yeast only 0.3 cc. of boiled yeast juice equivalent to 50 mg. of dry yeast was employed for the test. The results show that phosphorylation of vitamin B_1 by the mammalian organism is of vital importance as this co-enzyme plays an important rôle in the decarboxylation and dehydrogenation of pyruvic acid.[8] Furthermore animal tissues contain all of the vitamin B_1 as the pyrophosphoric ester.[8]

9635

Alleged Convulsant Properties of Brain Extracts.*

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Kroll[1] made the claim that convulsions could be produced in normal animals by the intravenous injection of extracts of brains removed from convulsing animals. Brain extracts from normal animals were said to be without convulsant properties. Both cats and rabbits were used but the former were regarded as more satisfactory. The minced brains were extracted 3 times with acetone and the remaining residue suspended in saline, extracted and filtered. This saline extract was employed in the experiments, the acetone extract being discarded.

Holmes[2] repeated these experiments on rabbits but used the acetone fraction of the extract. The acetone was evaporated and the remaining residue suspended in saline for administration. Such extracts were found to have marked constitutional effects when injected into animals. Death frequently occurred either with or without preceding convulsions. Extracts of normal brain as well as those from the brains of convulsing animals sometimes produced convulsions and both were equally lethal.

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[8] Lipmann, F., *Enzymologia*, 1937, **4**, 65.

* We thank Miss Doris Brophy for her assistance with these experiments.

[1] Kroll, F. W., *Z. f. d. ges. Neur. u. Psych.*, 1932-33, **143**, 780.

[2] Holmes, E., *J. Physiol.*, 1935, **85**, 400.

Despite the startling nature of Kroll's claim and the obvious pitfalls in the use of such crude extracts the subject merited investigation especially since Holmes used a different extract from that used by Kroll. Furthermore recent knowledge of explosive nervous activity mediated by chemical substances has made it necessary to think in such terms.

We have repeated the experiments using both types of extract. Cats have been used for the most part both as donor and recipient animals. Rabbits were used in a few instances as recipients. Our method of producing convulsions in the donor animals differed somewhat from that of the previous workers. They exposed the cerebral cortex under general anesthesia and stimulated electrically. In our experiments the unanesthetized animal was given a shock of 110 volts from the lighting main, one needle electrode being placed in the periosteum at the base of the skull and the other clipped to the lower lip. Such stimulation for 3 to 5 seconds is followed by a convulsion lasting several minutes, at first tonic and later clonic though the pattern varies. In 2 instances violent convulsions were produced by intravenous injection of thujone (1% by volume) to donor animals.

During progress of the induced convulsions the unconscious animal was bled through the carotids. Control animals were killed by intracardiac injection of air and similarly bled or simply bled under light ether anesthesia. The brain was removed at once, and ground thoroughly with sand in 80 cc. of acetone followed by 5 minutes' stirring with an electrical device. The acetone was then decanted and the remaining solid residue similarly treated with fresh acetone until thrice extracted.

Saline Extract of Residue (Kroll)—The stringy, solid residue remaining after acetone extract had been decanted off was dried by air blast, suspended in 60 cc. normal saline, stirred for 5 minutes and filtered twice through filter paper before use.

Acetone Extract (Holmes)—The three acetone extracts (240 cc.) were pooled and the acetone distilled off under negative pressure at 45°C. The remaining fatty residue was suspended in 60 cc. normal saline and traces of acetone removed by a bubbling air current.

Results. Extracts were made of the brains (wt. 20-25 gm.) of 10 cast, 2 of which were control animals and 8 of which had been convulsed in various ways. Twenty-five injections of brain extract were made intravenously into 19 animals (15 cats, 4 rabbits) in attempts to produce convulsions. The dose employed for rabbits was 1 to 2 cc. and that for cats 3 to 10 cc. Following 6 of these injections

TABLE I.

Donor					Recipient				
Cat. No.	Convulsive Agent	Type of Extract	Age of Extract, Minutes	Dose of Extract, cc.	Animal	Weight, Kg.	General Symptoms	Convulsive Symptoms	Death
1	110 volts 4 secs.	I	90	5	Cat		+++	±	—
		I	52	1	Rabbit		—	—	—
		I	124	2	,, (same)		—	—	—
		I	60	2	Rabbit		+++	±	+
2	None. Control light ether	I	100	5	Cat		++	—	—
		I	130	5	,,		++	—	—
		I	110	2	Rabbit		+++	—	+
		I	120	1	,,		+++	—	+
3	110 volts 7 secs.	I	135	5	Cat		—	—	—
			150	10	,, (same)		+++	—	+
4	110 volts 1 sec.	I	89	5	,,	2.90	++	—	—
5	110 volts 3 secs.	I	89	5	,,	2.05	++	±	—
6	Thujone 2 cc.	I	95	5	,,		+	—	—
			107	3	,, (same)		+	—	—
7	Thujone 1.8 cc.	I	95	5	,,	2.65	±	—	—
8	None. Control air in heart	I	94	10	,,	3.00	++	—	—
		II	163	7	,,	3.00	—	—	—
		III	199	5	,, (same)	3.00	+++	—	—
		III	174	3½	,,	2.65	±	—	—
		II	182	10	,, (same)	2.65	—	—	—
9	110 volts 4 secs.	I	97	9	,,	2.65	+++	±	—
		III	135	3½	,,	2.70	++	±	—
		II	213	7	,,		++	±	—
10	Faradic 15 mins. 110 v. 3 secs.	I	75	8	,,	3.10	+	—	—
		I	82	5	,, (same)	3.10	++	—	—

Extract I = Saline of brain residue (Kroll).
II = Acetone residue filtered.
III = Acetone residue unfiltered (Holmes).

there were slight convulsive movements only. In the remaining 19 there were no convulsions whatever. When convulsive movements did occur they consisted of brief tonic spasms lasting 5 to 15 seconds followed by irregular clonic or running movements of equally short duration. Twenty of the 25 injections resulted in marked generalized symptoms consisting of collapse, flaccidity, dilatation of pupils, involuntary micturition and defecation and apparent unconsciousness. At the height of this reaction there was stoppage of respiration and absence of heart sounds followed by rapid, panting respiration and slow, irregular heart sounds. Recovery usually occurred in about 15 minutes. Two rabbits died following injection of normal brain extract and one rabbit and one cat after injection of convulsed brain extract. There were no convulsions in any of these animals prior to death. Death occurred 2 to 3 minutes after injection.

Holmes investigated the effects produced by injection of the acetone fraction into a rabbit and gave excellent tracings of the alterations in heart rate, blood pressure and respirations. Perfusion of isolated hearts with Ringer's solution containing small amounts of extract caused cardiac depression and arrest. He concluded that such extracts have no direct effect on the central nervous system and that they cause symptoms and death through their depressant effect on the heart and circulation. We have repeated this experiment on the anesthetized cat using the saline residue fraction (Kroll) for injection. This fraction also causes cardiac slowing, arrest and irregularity associated with fall of blood pressure and depression of respiration. This was true of brain extracts from control and from convulsed animals. The acetone fraction (Holmes) is more potent in this respect, however.

These experiments, therefore, offer no support for the claim that extracts prepared in this manner contain a convulsant substance won from the brain during a convulsion. It should be stated, however, that a convulsing substance might be present in amounts unrecognizable because of the general constitutional effects of the extracts. Furthermore if convulsions were associated with the appearance of some convulsive substance in the brain it seems probable that the substance would appear with explosive rapidity and disappear quickly. The substance might thus have vanished during the 10 or 15 minutes required to remove the brain for fixation in experiments such as these.

Summary. Saline extracts of the brain residue remaining after acetone extraction and also the acetone fraction were prepared from normal cats and from cats undergoing convulsions due to thujone or electrical stimulation. Such extracts, when injected into normal cats or rabbits, caused marked constitutional symptoms due to cardiac depression but were without specific convulsant effect on the central nervous system. The claims of Kroll were therefore not substantiated.

9636

The Cure of Canine Blacktongue with Nicotinic Acid.*

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It has been reported by Elvehjem and coworkers[1] that canine blacktongue produced by subsistence on a diet of the Goldberger type is cured dramatically by administration of nicotinic acid. When this communication appeared we had 2 dogs exhibiting chronic blacktongue produced by maintenance on a slight modification of Goldberger's diet No. 123.[2] The modification consisted in the omission of the cowpeas and use of a slightly greater amount of casein. The disease, as exhibited by our animals, was characterized by poor appetite, progressive loss of weight, vermilion bands on the upper lips, general reddening of the oral mucosa, and, in one of the dogs, by persistent diarrhea. The vermilion bands on the lips and the reddening of the oral mucosa disappeared and reappeared repeatedly.

Presumably, the reason that the disease took a chronic rather than the acute form more commonly observed when using the Goldberger diets was the presence of a greater than usual amount of the antiblacktongue factor in the particular sample of corn used in our ration. This modified diet had been previously found to produce acute blacktongue.[3]

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* The expenses of this study were defrayed by a grant from the Rockefeller Foundation.

† Vitamin Research Fellow.

[1] Elvehjem, C. A., Madden, R. J., Strong, F. M., and Wooley, D. W., *J. Am. Chem. Soc.*, 1937, **59**, 1767.

[2] Goldberger, J., Wheeler, G. A., Lillie, R. D., and Rogers, L. M., *U. S. Public Health Rep.*, 1928, **43**, 657.

[3] Street, H. R., Doctor's thesis, 1936, University of Minnesota.

The 2 dogs were treated with nicotinic acid hydrochloride obtained from the Eastman Kodak Company. It was given by mouth, the dosage being approximately 5 mg. per kilo of body weight per day.

There was a prompt resumption of normal appetite in both dogs and an immediate and sustained increase in body weight. The vermilion bands on the upper lips and the reddening of the oral mucosa disappeared and did not return. The watery diarrhea exhibited by one of the dogs disappeared. The body weight curves are shown in Fig. 1.

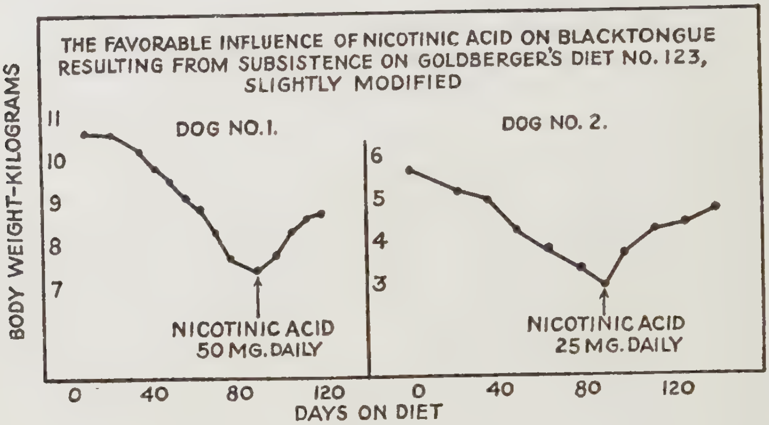

Fig. 1.

Conclusion. The finding that nicotinic acid corrects the deficiency of the Goldberger blacktongue-producing diet is confirmed.

9637

Alteration in the Immunological Specificity of Fibrinogen by the Action of Fibrinolysin of the Hemolytic Streptococcus.

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It has been shown by Garner and Tillett[1] that if fibrinogen is incubated with hemolytic streptococcus fibrinolysin for a brief period, the fibrinogen can no longer be changed to fibrin upon the addition of thrombin. However, these authors came to the conclusion that although changes do take place in the fibrinogen when acted upon by the fibrinolysin, the degradation of the molecule is not great. In further studies Garner[2] added that the end product of the reaction could not be differentiated, by immunological experiments, from the original fibrinogen solution.

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[1] Garner, R. L., and Tillett, W. S., *J. Exp. Med.*, 1934, **60**, 255.

The possibility of an antigenic alteration of fibrinogen by fibrinolysin was further studied in the following manner.

Human fibrinogen was prepared according to the method outlined by Tillett and Garner,[3] except that the fibrinogen solution was purified by precipitating with 50% saturation with NaCl. It was then centrifuged and the precipitate dissolved in distilled water. The fibrinogen was again precipitated out and dissolved as above, the procedure being repeated 7 times. The fibrinogen solution was then tested by the precipitin reaction against anti-human globulin serum.* The reaction was very faint, indicating a minimum amount of contaminating globulin. This fibrinogen was used in immunizing rabbits. The immune serum produced was filtered through a Berkefeld filter and then inactivated at 56°C. for a half-hour. This inactivation was necessary because the serum contains a small amount of thrombin, and when the fibrinogen is added in the precipitin test a small amount of reactive fibrin is formed which interferes with the test. Inactivating the serum removes this difficulty, as was shown by inactivating normal serum and adding fibrinogen.

Precipitin tests were carried out using a constant amount of antiserum and varying amounts of fibrinogen, and the same amounts of fibrinogen-fibrinolysin solution. Thus the tests were run in two series:

(a) Control Solution: 1 cc. broth (0.05% dextrose) + 2 cc. fibrinogen, incubated for 24 hours prior to the precipitin tests with the antiserum.

(b) Fibrinogen-fibrinolysin Solution: 1 cc. fibrinolysin + 2 cc. fibrinogen, incubated for 24 hours prior to the precipitin tests with the antiserum.

The fibrinogen contained 0.261 mg. nitrogen per cc.; the fibrinolysin was the filtrate of a 20-hour 0.05% dextrose broth culture of hemolytic streptococcus No. 260, obtained from a case of acute pharyngitis. The fibrinolysin lysed the clot of human fibrinogen and thrombin in less than 20 minutes. Varying amounts of solutions (a) and (b) were added to 1 cc. samples of the rabbit-anti-human fibrinogen serum. The tubes were incubated for one hour at 37°C., and were then placed in the ice-box overnight. The precipitates formed were then washed in distilled water and then diluted to ten cc. with distilled water and analyzed for their total nitrogen content by the Kjeldahl method. The results of a representative analysis are shown in Tables I and II.

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[2] Garner, R. L., *J. Biol. Chem.*, 1935, **109**, xxxvi.

[3] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

* This anti-human globulin serum was kindly furnished by Dr. Forrest E. Kendall of the Research Division for Chronic Diseases, Department of Hospitals, Welfare Island, New York.

TABLE I.
Using Control Solution (a) above.

	Total N of fibrinogen Solution (a) mg.	Total N in Precipitate mg.
1. 1 cc. anti-serum + 1 cc. Solution (a)	.174	.31
2. ” ” + 0.75 ” ” ”	.130	.33
3. ” ” + 0.50 ” ” ”	.087	.30
4. ” ” + 0.25 ” ” ”	.043	.21

TABLE II.
Using Solution (b) above.

	Total N in Precipitate mg.
1. 1 cc. anti-serum + 1 cc. Solution (b)	.075
2. ” ” + 0.75 ” ” ”	.075
3. ” ” + 0.50 ” ” ”	.072
4. ” ” + 0.25 ” ” ”	.072

From the above results it appears that the fibrinolysin does change the specificity of the fibrinogen, as is evidenced by the change in the quantity of the precipitates when fibrinolysin-fibrinogen mixtures are tested with anti-fibrinogen serum. The next step was to investigate the rapidity of the action of fibrinolysin in bringing about this change in the fibrinogen.

Streptococcus R333, obtained from a case of streptococcus pneumonia, was grown in 0.05% dextrose broth. The filtrate lysed the clot of human fibrinogen and thrombin in 6 minutes. This fibrinolysin was then incubated with fibrinogen at 37° C. At intervals of 15 min., 30 min., 45 min., 1 hr., and 2 hr. samples were withdrawn and added to 1 cc. quantities of the rabbit-anti-human fibrinogen serum. The amount of fibrinogen-fibrinolysin withdrawn was always equivalent to 0.087 mg. of fibrinogen nitrogen; this amount was slightly less than the amount necessary to bring about maximum precipitation, as was shown in the previous experiment. After incubation the tubes were placed in the ice-box over night, and the following day the precipitates were analyzed. The results are presented in the graph, Fig. 1.

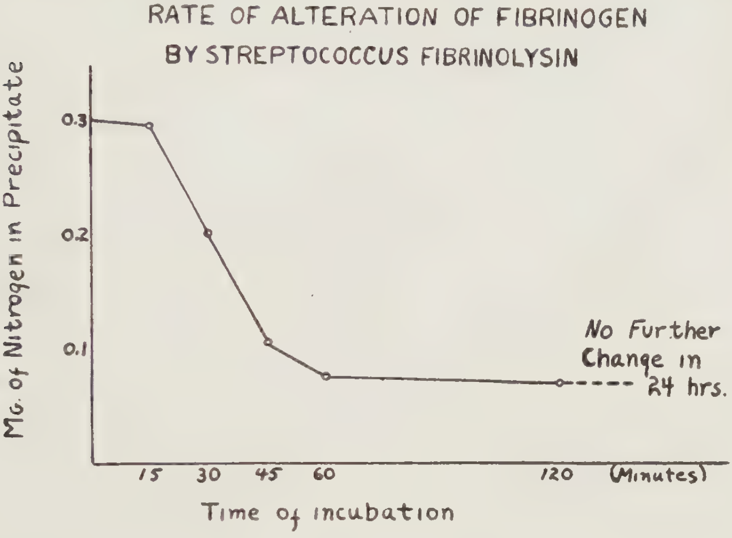

FIG. 1.

The results indicate that there is an initial lag period which is followed by a rapid activity of the fibrinolysin on the fibrinogen, as is shown by the decrease in the amount of the specific precipitate. The reaction is complete in little more than one hour, and there is no further degradation even in 24 hours.

In the work of Garner and Tillett[1] the fibrinogen used probably contained a considerable amount of globulin. This is indicated because in their work when they incubated the fibrinogen with fibrinolysin and then added $(NH_4)_2SO_4$ to 25% saturation they did not obtain any precipitate. However, when they increased the $(NH_4)_2SO_4$ to 35-45% saturation a precipitate was obtained. This latter amount of $(NH_4)_2SO_4$ will precipitate serum globulins. Probably no precipitate was obtained with 25% saturation with $(NH_4)_2SO_4$ because the fibrinogen had been altered and all that remained was the serum globulins. In the experiments completed above the fibrinogen was purified so that it contained only a minimum amount of globulin as was shown by testing with human anti-globulin serum. Fibrinogen prepared by the Tillett and Garner[3] method gave a much greater precipitate with the same anti-globulin serum, showing that it contained a not inconsiderable amount of globulin.

When the fibrinogen-fibrinolysin solution was incubated for from one to 24 hours the amount of precipitate formed, when the solution was added to the anti-fibrinogen serum, was almost constant. Considering the rapid and progressive change in the fibrinogen within the first hour of incubation with the fibrinolysin it is suggested that the substance remaining in solution, which reacts with the anti-fibrinogen serum, is either a split product of the fibrinogen resulting from the action of fibrinolysin or a small amount of serum globulin.

Another point of interest is the apparent lag period of 15 minutes before fibrinolysin lyses fibrinogen as indicated on the graph. This, however, probably has no significance if we consider it in the following manner:

Assuming that all or most of the antigen nitrogen is present in the precipitate, Table I can be presented as shown in Table III.

TABLE III.

Antigen N mg.	N in precipitate mg.	Antibody N mg.
.174	.31	.136
.130	.33	.200
.087	.30	.213
.043	.21	.167

It appears possible that the maximum antibody nitrogen may have been precipitated by the addition of .065 mg. of fibrinogen nitrogen. Thus the first 15 minutes is not actually a lag period, but it represents the time taken to reduce the amount of fibrinogen to the point where it will precipitate the maximum amount of antibody.

Summary. 1. A fairly pure fibrinogen was prepared and used to immunize rabbits. 2. Precipitin tests were carried out with the anti-serum formed; the precipitinogens used were fibrinogen alone and fibrinogen + fibrinolysin. 3. Analyses of the precipitates indicate that the fibrinolysin does have an effect on the fibrinogen which alters its antigenic specificity. 4. The action of the fibrinolysin is very rapid, and the change it produces in the fibrinogen is accomplished in little more than one hour.

9638

Effect of Certain Nutritional Deficiencies on Various Phosphorus-containing Fractions of the Chick Brain.*

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In a series of studies designed to investigate the lipids of the brain in nutritional deficiencies it became apparent that a metabolic disturbance occurred which resulted in a variation in the phosphorus distribution. The early work dealt mainly with tissues from animals suffering from deficiencies of the B complex. The work of Sure and Buchanan[1] had indicated that a disturbance of the blood serum phosphatase occurred in the vitamin B complex deficiencies. It seemed worth while to make a more extensive study of the brain phosphorus of chicks on diets low in certain essential factors.

The nutritional deficiencies were produced in our laboratory in collaboration with Elvehjem and associates. The following deficiency conditions were studied: vitamin A, vitamin B_4, and encephalomalacia. Chicks of the same age, on a ration known to be adequate in all these factors, were sacrificed as controls. The chicks with typical deficiency symptoms were killed at the age of 10-21 days by bleeding through the carotid artery. The brains were immediately removed and sliced mid-sagitally; half of the brain was preserved for histologic study, the other half was placed in a drying oven at 56°-58°C. for 48 hours.

The method of extraction of the dried brains was essentially that of Bloor,[2] using hot ethyl alcohol and repeated extractions. The brains were ground, extracted repeatedly with hot alcohol and the extracts were decanted and made up to volume in 100 cc. flasks. No inorganic phosphorus was detected in the extracts, therefore only the total phosphorus was determined on suitable aliquots. The brain residue was dried and weighed. The phosphorus was partitioned according to the principle described by Hawk and Bergeim[3] and determined by the method of Fiske and Subbarow.[4] The residue after alcoholic extraction was extracted with trichloracetic acid.

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* Published with the permission of the director of the Wisconsin Agricultural Experiment Station.

[1] Sure, B., and Buchanan, K. S., *Am. J. Digest. Dis. and Nutr.*, 1936, **3**, 493.

[2] Bloor, W. R., *J. B. C.*, 1929, **82**, 273.

[3] Hawk and Bergeim, *Practical Physiological Chemistry*, 11 ed., 1937, 460.

[4] Fiske, C. H., and Subbarow, Y., *J. B. C.*, 1925, **66**, 375.

TABLE I.
Phosphorus Content of Chick Brain.

Disease	Total No. of chicks used	Total acid-soluble P in Ccl_3COOH extract (mg. %)		Inorganic P in Ccl_3COOH extract (mg. %)		Ester P in Ccl_3COOH extract (mg. %)		% alcohol extract		P in alcohol extract (mg. %)	
		Range	Ave.	Range	Ave.	Range	Ave.	Range	Ave.	Range	Ave.
Controls	31	3.6-6.6	4.9	2.4-3.7	3.0	0.3-3.7	2.0	39.3-51.4	47.6	2.4-10.5	6.3
A-deficient	24	3.1-7.2	4.8	3.0-4.6	3.7	trace-3.2	1.0	42.3-54.0	47.8	4.0-14.1	8.1
B_4 ,,	29	3.1-6.2	4.3	2.3-4.4	3.5	trace-2.2	0.8	43.6-57.0	47.5	3.3-12.1	7.6
Encephalomalacia	24	3.0-6.4	4.5	2.8-4.9	3.5	trace-3.0	1.0	44.6-53.2	48.8	3.5-11.0	7.2

Inorganic phosphorus and total acid soluble phosphorus were determined on suitable aliquots of this extract and the difference between the two was used as a measure of the ester phosphorus. The residue remaining after alcoholic and trichloracetic acid extractions was digested (wet ash) and the total phosphorus determined.

Table I contains the condensed results of these studies. Inspection of the table indicates that the ester phosphorus was considerably reduced in the nutritional deficiencies. The greatest reduction occurred in vitamin B_4 deficiency. It was also noted that the inorganic phosphorus was increased, but the increase was not equal to the amount of reduction in the ester phosphorus. Consequently, the total acid soluble phosphorus was lowered.

The phosphorus content of the alcoholic extract was slightly increased in the deficiency diseases as compared to the values observed in the controls. The phosphorus in the residue of the brain after alcoholic and trichloracetic acid extraction was apparently unaffected and contained from 1.2-1.3 mg. % for all groups. No difference was noted in the quantity of the alcohol-soluble fraction of the normal chick brain as compared to the deficient brains. The total phosphorus content of the brain of the deficient chicks was, however, equal to that of the control chicks and contained from 12.1 to 13.1 mg. %. Thus it appears that a vitamin A deficiency, encephalomalacia, or B_4 deficiency causes a disturbance in the phosphorus distribution in the chick brain.

The reduction in ester phosphorus in the brains of the nutritional deficiencies studied appears to be of importance. In 35% of the cases the amount of ester phosphorus calculated to be present in vitamin A or B_4 deficiency, or in encephalomalacia was *nil* or exceedingly low. Coincident with the lowering of ester phosphorus was an increased inorganic phosphorus. These findings indicate a disturbance in phosphorus metabolism. It is possible that phosphorylation does not occur, or if so, it proceeds at a greatly reduced rate, or dephosphorylation occurs at an excessive rate.

Summary and Conclusions. The various fractions of chick brain, namely, the alcohol extract fraction, the trichloracetic acid extract fraction, and the fraction remaining after both extractions, were analyzed for their phosphorus content. In comparing the values obtained from chicks on a normal ration with those obtained from chicks receiving rations low in one of the essential factors (vitamin A, vitamin B_4, and encephalomalacia) the following results were obtained.

The ester phosphorus of the trichloracetic acid fraction was markedly reduced in the deficiency diseases, while the inorganic phosphorus was increased but not to the same extent as the former was reduced. The alcohol extract phosphorus fraction was only slightly increased. The phosphorus of the brain residue after alcohol and trichloracetic acid extractions as well as the total phosphorus content of brain was unaffected. Further, the amount of alcohol-soluble material of the brains was similar in both deficient and control chicks. Certain nutritional deficiencies, among them the lack of the encephalomalacia factor, vitamin A, or B_4, cause a disturbance of normal phosphorus metabolism in the brain of the chick.

9639 P

Relation of the Carrier State to Pneumococcal Peritonitis in Young Children with the Nephrotic Syndrome.

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Most deaths in children afflicted with the nephrotic syndrome are caused by intercurrent infection. In nearly every instance, the infection is due to the pneumococcus, and the site of localization is the peritoneal cavity.

In the nephrotic child peritonitis develops suddenly, and without any apparent precipitating factor such as an upper respiratory infection. This fact suggests two possibilities: First, that peritonitis is due to the acquisition from a carrier of an invasive strain of pneumococcus, and second, that peritonitis is caused by the strain or strains of pneumococcus which are carried in the patient's nasopharynx.

Nose and throat cultures have been made periodically on 12 nephrotic children, 1½ to 7 years of age. The children have been observed over periods varying from one week to 2 years. The pneumococci isolated from the nasopharynx have been typed by Neufeld's technic. Only those studies are presented here which deal with the children developing peritonitis.

In every child who developed peritonitis, the same type of pneumococcus has been recovered from the throat and the peritoneal cavity. During the carrier-state repeated cultures showed the same type or types of pneumococcus to persist in the nasopharynx. In 2

TABLE I.

Case	Age, yrs.	Sex	Type of pneumococcus causing peritonitis	Type of pneumococcus in patient's nasopharynx	Time of isolation of organism from throat in relation to onset of peritonitis	Outcome
1. R.R.	3	M	XIX	XI and XIX	69 days before peritonitis developed	Died
2. J.Mc.	7	M	X	X	47 days before peritonitis developed	,,
3. R.B.	2	M	VI	IV and VI	7 days before peritonitis developed	,,
4. J.F.	6	M	XX	XX	Day on which peritonitis developed	,,
5.* J.C.	4	M	XIX	XIX	a. Day on which peritonitis developed	Recovered
					b. 77 days after peritonitis developed	
6. L.E.	3	F	XXVIII	XXVIII	17 days after peritonitis developed	,,

*This case was in contact with Case 1 for several weeks before the latter developed Type XIX pneumococcal peritonitis.

such cases 2 types of pneumococcus were carried persistently in the nasopharynx, but one type only was isolated from the peritoneal cavity during the acute infection. That the carrier-state may continue for a prolonged time following recovery from peritonitis is shown by the isolation of the infecting type of pneumococcus from the throat of 2 convalescent cases.

The data on 6 children with the nephrotic syndrome who developed pneumococcal peritonitis are presented in Table I. This shows the time at which the type of pneumococcus which caused peritonitis was isolated from the throat, in relation to the time of onset of the peritoneal infection.

In 3 cases (1, 2, 3) the type of pneumococcus which caused the peritonitis was isolated repeatedly from throat cultures taken over a variable period before peritonitis developed (69, 47, and 7 days respectively). In 2 cases (4, 5) the invading type of pneumococcus was recovered from throat cultures taken within a few hours after the onset of peritonitis and in one of these cases (5) the organism was isolated repeatedly from the throat during a period of 77 days after recovery from peritonitis. In one case (6) the organism causing the peritonitis was not recovered from the throat until 17 days after the subsidence of the acute infection. The correlation noted in the present study between the types of pneumococcus found during the carrier-period and during peritonitis was not observed in the cases reported by Peacock and Werner.[1]

Summary. In 6 young children with the nephrotic syndrome who developed pneumococcal peritonitis, the type of pneumococcus recovered from the nasopharynx was the same as that causing peritonitis. Evidence is presented which indicates that peritonitis may be caused by a strain of pneumococcus of the same specific type as that of the organism known to have been carried in the nasopharynx for a considerable time before the development of the infection; this carrier-state may persist for a prolonged period after recovery from peritonitis.

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[1] *Am. J. Dis. Child.*, 1937, **53**, 1022.

9640 P

Toxicity of Sulfanilamide and Diethylene Glycol.

Charles F. Poe and Paul C. Witt.

From the Department of Chemistry, University of Colorado, Boulder, Colorado.

Sulfanilamide has gained some prominence in the treatment of various infections caused by the gonococcic, meningococcic, and streptococcic organisms. However, the recent deaths caused by the administration of an elixir made from this drug dissolved in diethylene glycol has cast some doubt on its availability as a therapeutic agent. Some 75 deaths have been reported from the use of this so-called elixir.

Toxicity tests were conducted separately with the sulfanilamide and diethylene glycol by using white rats approximately 4 weeks old and weighing from 45 to 60 gm. A mixture of these two substances was also used in the tests. Oral and intraperitoneal administrations were used.

The toxic dose of the diethylene glycol in the preliminary experiments was found to be around 12,500 mg. per kilo of body weight when given intraperitoneally. The oral doses to produce death were approximately the same. Recently, several investigators[1, 2, 3] have found diethylene glycol to be toxic for small animals. The toxic doses of the sulfanilamide were approximately 2600 mg. per kilo of body weight.

An elixir was prepared containing the same amounts of the two principal ingredients as the Elixir of Sulfanilamide-Massengill.[4] This elixir contained 40 grains of sulfanilamide per ounce of a solvent containing 72% diethylene glycol by volume. The results obtained with the elixir paralleled very closely those reported by Geiling, Coon, and Schoeffel.[5] The heating of the elixir for 30 minutes at 95°C. did not change the toxicity. The elixir containing given amounts of sulfanilamide appeared to be more toxic than the sulfanilamide alone. Various doses up to 200 mg. of sulfanilamide per kilo were given orally 3 times a day for 10 days. The results obtained confirmed those of the above-mentioned investigators.

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[1] Hanzlik, P. J., Seidenfeld, M. A., and Johnson, C. C., *J. Pharm. and Exp. Therap.*, 1931, **41**, 387.

[2] Haag, H. B., and Ambrose, A. M., *J. Pharm. and Exp. Therap.*, 1937, **59**, 93.

[3] Holck, H. G. O., *J. Am. Med. Assn.*, 1937, **109**, 1517.

[4] Editorial, *J. Am. Med. Assn.*, 1937, **109**, 1367.

[5] Geiling, E. M. K., Coon, J. M., and Schoeffel, E. W., *J. Am. Med. Assn.*, 1937, **109**, 1531.

In order to render the sulfanilamide more soluble, molecular quantities of hydrochloric acid were added to the sulfanilamide and the mixture was heated for 2 minutes. Various amounts of this hydrochloride were injected intraperitoneally into white rats. The results are given in Table I.

TABLE I.
Toxicity of the Hydrochloride of Sulfanilamide When Administered Intraperitoneally.

Dose mg./kg.	Male Rats		Female Rats		% Mortality
	Lived	Died	Lived	Died	
200	1	0			
300	1	0			
350	5	2			28
400	5	2	3	1	27
450	4	2	4	2	33
500	5	2	4	2	31
600	1	2	2	1	50
700	1	1			50
800	0	3	1	2	83
900	0	2	1	2	80
1000	0	1	0	1	100

The outstanding conclusion to be drawn from the results in Table I is that the hydrochloride is about 4 times as toxic as the base. These results may be explained on the basis of the increased solubility of the hydrochloride.

9641 P

Bodily Electrical Potential Changes Associated with Ovulation and Early Pregnancy in the Chimpanzee.*

GLEN FINCH, ROBERT M. YERKES AND JAMES H. ELDER.
From Yale Laboratories of Primate Biology.

Recently Burr and associates[1] discovered that ovulation in the rabbit is accompanied by large increases in electrical potential. Observations on women, using the apparatus and technique described by Burr, Lane, and Nims,[2] have been reported by Burr and Musselman.[3] Applications of the vacuum tube potentiometer have been reported also by Reboul, Friedgood, and Davis,[4] and by Rock, Reboul, and Wiggers.[5] The present preliminary report offers the results of an attempt to apply the Burr-Lane-Nims technique to the study of ovulation in the chimpanzee.

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* The writers gratefully acknowledge the assistance of the Committee for Research in Problems of Sex, National Research Council, and also that of their colleague, Doctor H. S. Burr.

[1] Burr, H. S., Hill, R. T., and Allen, E., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 109.

[2] Burr, H. S., Lane, C. T., and Nims, L. F., *Yale J. Biol. Med.*, 1936, **9**, 65.

A sexually mature chimpanzee (No. 4 in the laboratory series), with regular menstrual cycles, was trained to present the backs of her hands for the application of salt-bridge electrodes and to remain passive during the course of a series of potential difference measurements. Measurements were taken daily over a period of several weeks.

The curve of Fig. 1 shows the potential measurements in millivolts through a normal sexual cycle (April 6 to May 6). Each voltage point represents the median of 10 or more determinations, with the exception of days 5, 10, and 12. On these days there were 4, 2, and 4 observations respectively. Occasional refusal of the subject to coöperate or temporary difficulties with the apparatus account for the omission of data for certain days. The menstrual history of our subject and previous results of the controlled mating method[6] enabled us to predict that ovulation should occur in this subject on the 16th or 17th day. Accordingly, on days 16, 17, and 18 one or two additional series of observations were made. The sexual status of the subject is represented below the voltage curve. The line following the 4 days of menstruation represents the relative amount of genital swelling. The heavy portion of this line indicates the period of maximal swelling. General description of the sexual cycle of the chimpanzee has been given by Elder and Yerkes.[7]

In contrast with the small variations in potential which appear from day to day, the median for the 17th day is higher than any of those before or after that date. The magnitude of this maximum value is not as great as might be expected, but it occurs at the time predicted, and also, if it is indeed a phenomenon associated with ovulation, stands in appropriate relationship to the beginning of detumescence. All of our isolated fertile matings fall within the last 6 days of maximal genital swelling.

Fig. 2 presents similar data for the next cycle of the same female. A maximum value again appears on the 17th day, followed by an-

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[3] Burr, H. S., and Musselman, L. K., *Yale J. Biol. Med.*, 1936, **9**, 155; Burr, H. S., Musselman, L. K., Barton, D. S., and Kelly, N. B., *Science*, 1937, **86**, 312.

[4] Reboul, J., Friedgood, H. B., and Davis, H., *Am. J. Physiol.*, 1937, **119**, 387.

[5] Rock, J., Reboul, J., and Wiggers, H. C., *New Eng. J. Med.*, 1937, **217**, 654.

[6] Elder, J. H. [The time of ovulation in chimpanzees], in press.

[7] Elder, J. H., and Yerkes, R. M., *Anat. Rec.*, 1936, **67**, 119.

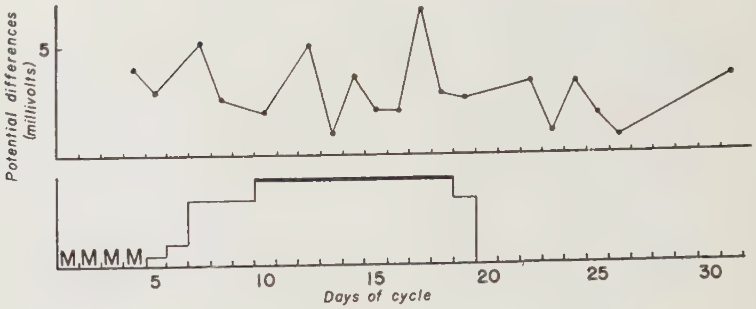

FIG. 1.

Electrical potential changes during one menstrual cycle in a chimpanzee. Lower curve shows the relative size of genital swelling, the heavier portion representing the period of maximal swelling.

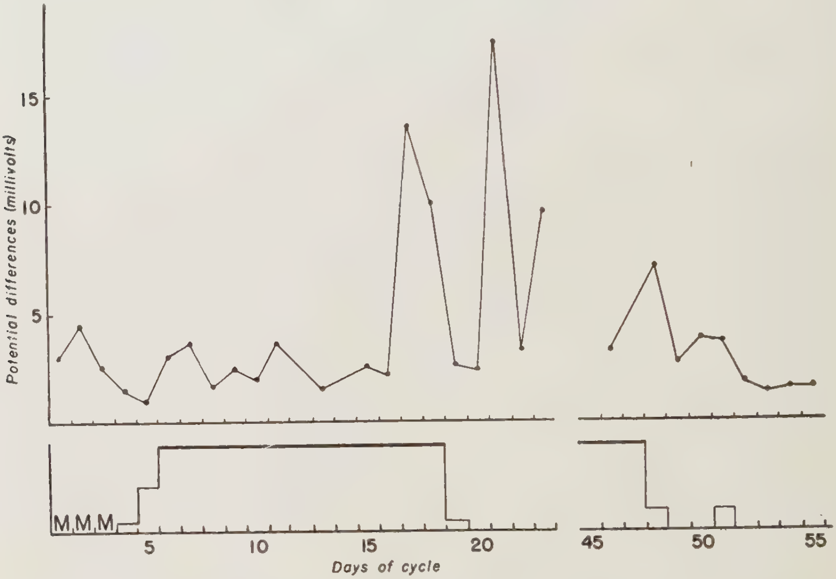

FIG. 2.

Electrical potential changes in the cycle succeeding the one represented in Fig. 1. The second peak in the curve is of particular interest because of a subsequent diagnosis of pregnancy.

other peak 4 days later. This second rise in the curve is of particular interest because of a subsequent diagnosis of pregnancy confirmed by a series of Friedman tests. Because pregnancy was not suspected at the time that the high readings were obtained on days 21-23, the apparatus was completely dismantled for checking, and a period of 22 days elapsed before it was reassembled and put into operation again. We are inclined to interpret the high potentials of the 17th day as concomitant with ovulation, while those of the 21st day may be correlated with fertilization and early pregnancy changes.

These results indicate that the Burr-Lane-Nims technique may prove valuable for the early diagnosis of pregnancy as well as for the detection of ovulation in the chimpanzee.

9642

Therapy of Experimental Staphylococcus Infections with Sulfonamide Compounds.

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Staphylococcus infections either in man or in experimental animals have not yielded to treatment with sulfanilamide. However, Hörlein* has apparently reported successful results against this organism with "Di-Septal," a dimethylated derivative of di-sulfanilamide. The latter compound appears to have been first synthesized by Rosenthal,[1] who reported on its use in pneumococcal infections in mice. This communication represents a preliminary attempt to compare the efficacy of these 2 compounds with sulfanilamide, in experimental staphylococcus infections in mice and in man.

In the following mouse experiments, all infections were established by giving 0.2 cc. of a 20-hour, undiluted broth culture of an hemolytic *Staphylococcus aureus* into the tail vein. This strain (our No. 451) was isolated in this laboratory from a human case, and was virulent for mice. All medication was given daily by the oral route and consisted of 20 mg. of the drug suspended in 0.25 cc. of 15% gum acacia solution. Medication was continued for 15 successive days in each series, and in the case of those receiving immediate therapy was started on the day that the infecting dose was given. In the delayed therapy groups 3 days elapsed between infection and the institution of medication.

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* In a paper read before the British Association for Advancement of Science, August, 1937. The information available to us refers to its use against staphylococci and the Welch bacillus, as well as hemolytic streptococci. Staphylococcal osteomyelitis was mentioned in connection with its favorable action.

[1] Rosenthal, S. M., Bauer, H., and Branham, S. F., *Pub. Health Rep.*, 1937, **52**, 662.

Series I. The objective of this series was an evaluation of the *protective* effect of the 3 compounds. It consisted of 84 mice divided into 4 groups of 21 each. All were infected; one group was kept as a control; one group received sulfanilamide; one group received di-sulfanilamide, and the remaining group received di-methyl-di-sulfanilamide (D 373 Winthrop†). Table I shows the percent dead in each group at 2-day intervals. Animals surviving 32 days were killed and counted as survivors. In all these groups treatment was started immediately and continued for 15 days.

TABLE I.

Compound	No. mice	% dead at, days														% Survivals
		2	4	6	8	10	12	14	16	18	20	22	24	26	28	
Controls	21	0	19	29	29	33	43	52	62	67	71	71	71	76	86	14
Sulfanilamide	21	5	19	33	43	48	48	52	52	57	57	57	62	62	62	38
Di-sulfanilamide	21	0	5	5	10	14	14	14	14	14	14	19	29	33	38	62
Di-methyl-di-sulfanilamide	21	5	10	19	24	29	33	33	38	38	43	52	57	62	71	29

The pathological picture was uniform throughout the group, being characterized chiefly by miliary abscesses of the kidneys with only an occasional lesion of the liver or spleen. The histological studies on these tissues had not as yet been completed.

The following facts are apparent on the basis of the foregoing series: 1. On the basis of survivors, the effectiveness of the compounds falls in the following order: di-sulfanilamide, sulfanilamide, di-methyl-di-sulfanilamide. 2. On the basis of early deaths, sulfanilamide gives poorer results than do the controls. This may be due to the higher toxicity of the compound. 3. The continuation of deaths after the cessation of therapy may indicate that treatment should be continued for a longer period.

Series II. The objective of this series was an evaluation of the *therapeutic* effect of the drugs. The foregoing experiment was repeated with 141 mice. In this series non-infected mice receiving the same medication were introduced as drug controls. Di-sulfanilamide and di-methyl-di-sulfanilamide only were employed. The virulence of the infecting strain was increased for this series by 2 mouse passages. The infected mice receiving each drug were divided into 2 groups in one of which therapy was instituted immediately and in the other delayed for 3 days after infection. This period of delay gave ample time for the establishment of gross lesions in the kidneys. The results are reported in Table II.

It is apparent from Table II that: 1. In the early period there is no

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† Both this compound and the di-sulfanilamide were kindly furnished us by the Winthrop Chemical Company and the Alba Chemical Company.

TABLE II.

Therapy	No. mice	% dead at, days														% Survivals
		2	4	6	8	10	12	14	16	18	20	22	24	26	28	
Controls	33	0	15	36	48	61	64	67	76	79	79	79	85	88	88	12
Di-sulfanilamide (immediate)	22	0	0	14	23	23	27	36	36	36	41	41	45	59	64	36
Di-sulfanilamide (delayed)	22	0	14	18	23	32	32	32	32	41	41	41	50	59	59	41
Di-methyl-di-sulfanilamide (immediate)	22	0	9	18	59	59	59	59	59	59	64	64	86	91	91	9
Di-methyl-di-sulfanilamide (delayed)	22	0	9	14	68	68	68	77	86	100	—	—	—	—	—	0

really significant difference between the di-methyl-di-sulfanilamide and the controls. 2. The time at which therapy is instituted is not critical for either drug. 3. Di-sulfanilamide is definitely superior to di-methyl-di-sulfanilamide.

The pathological picture was essentially similar to that found in the first series, differing only in being more severe. The uninfected controls receiving the drugs gave no results of significance. There was slight indication, however, that the di-sulfanilamide might be the more toxic. Weight curves kept for each group showed that over the period of the first 7 days the drug controls lost 16%, the infected controls, 28%, the di-sulfanilamide treated, 20%, and the di-methyl-di-sulfanilamide treated, 24% in average weight.

Four cases of chronic staphylococcic osteomyelitis have been treated for a period of several weeks, first with "Di-Septal," and later with di-sulfanilamide. The most severely ill patient died, as was anticipated, showing no clinical response to the drug's action. The clinical course of the remaining 3 cases has not been noticeably altered by either drug. No signs of toxicity have appeared. A case of acute staphylococcic arthritis in a child was uninfluenced by di-sulfanilamide and after several days developed septicemia, followed by death.

Conclusions. Generally speaking, our experience with these drugs in staphylococcic infections is in marked contrast to the decisively favorable results of sulfanilamide in hemolytic streptococcal infections. Disulfanilamide, by virtue of its apparent therapeutic effect over a limited time period has a certain promise, which, however, failed of clinical confirmation in the small number of cases observed. A more diversified experimental trial is merited. Both experimentally and clinically the results with "Di-Septal" in this preliminary study were essentially negative.

9643

Luteinizing Hormone in Bird Hypophyses.*

Samuel L. Leonard.

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Since the follicle stimulating and luteinizing hormones have been shown to exist in mammalian hypophyses, an investigation to determine their presence in bird hypophyses seemed of interest. This would be of particular interest since the absence of corpora lutea in birds'[1] ovaries might lead one to suspect an absence of the luteinizing hormone in their hypophyses, when tested on mammals. An early observation led me to believe that bird hypophyses lacked the effective gonadotropic hormones for mammals because 10 fresh glands from 12-week-old chickens failed to induce ovarian changes or sexual precocity in an immature mouse.

More recently, Benoit,[1] working with duck hypophyses, and Witschi, Stanley and Riley,[2] working with turkey hypophyses, showed that the gonad-stimulating hormones from these birds were active in mammals. The results of similar studies on chicken hypophyses particularly with regard to the luteinizing hormone are reported here.

Chicken heads from a local butcher were collected at the end of each Saturday's kill during the spring of 1937. The hypophyses were removed and dropped into acetone and dried. Because of difficulties in obtaining material, no separation according to sex or age of the birds was made. In all, 2.5 gm. of powdered glands were obtained from approximately 2,000 birds.†

For testing, normal and hypophysectomized female rats and female rabbits were used. The powdered glands were injected as a watery suspension. Several different methods were used to detect the luteinizing hormones.

Pairs of immature female rats, 30 days old, received total doses of 10, 20, 30, 40, and 50 mg. of the powdered gland suspended in water. Three injections were made in 3 days, the animals killed

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* Part of the work was done at Union College, supported by a grant from the Rockefeller Foundation.

[1] Benoit, J., *C. R. Soc. Biol.*, 1935, **118**, 672.

[2] Witschi, E., Stanley, A., Riley, J., Proc. Soc. Exp. Biol. and Med., 1937, **36**, 647.

† The help of Mr. R. Royal and Mr. A. Riley in the laborious task of removing the glands is acknowledged with thanks.

24 hours after the last injection. The average ovarian weights obtained were 18.0, 28.3, 58.0, 53.4, and 39.8 mg. respectively (control 15 mg.). Follicular growth accompanied by oestrous changes in the uterus and vagina occurred in every case except in the 10 mg. dose and corpora lutea were found in the ovaries of rats receiving 30 mg. doses or higher. The loss of some material at the injection site at the highest dosage accounted for the drop in ovarian weight. These results, in general, are the same as obtained ordinarily with rat hypophyseal implants.

The augmentation test has been used to detect luteinizing hormone whereby in the presence of the follicle stimulating hormone, the luteinizing hormone will markedly increase the ovarian weight in immature rats and produce luteinized ovaries.[3] An extract of menopause urine was injected as the follicle-stimulating hormone (F.S.H.). In the first experiment (Table I), F.S.H. equivalent to 100 cc. of urine and 50 mg. of chicken hypophyses were used; in the second experiment, F.S.H. equivalent to 150 cc. and 30 mg. of chicken hypophyses were used. The results are given in Table I. Using 15 mg. as an established control ovarian weight, the gain in ovarian weight in the combined injections divided by the sum of the separate gains gave the percentage of augmentation. The resulting 155% and 222% augmentation, in addition to the luteinized ovaries obtained with the combined treatment, indicated the presence of luteinizing hormone in chicken hypophyses.

TABLE I.
Augmentation Test for Luteinizer.

	Ovarian Weights, mg.			
Exp.	F.S.U.	Chick A.P.	Combined	% Augmentation
1	15.0	25.5	36.3	
	16.0		29.0	155
			31.0	
2	29.4	21.3	56.3	
	31.3	23.0	49.2	222
			89.2	

The hypophysectomized rat ovary will respond to the luteinizing hormone, in the presence of an adequately grown follicle, by forming corpora lutea. Immature female rats were hypophysectomized and after a period of 12 to 14 days were injected with varying amounts of chicken hypophyses. The results are given in Table II. The vaginas of all injected rats were opened on the fourth day in the

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[3] Leonard, S. L., *Endocrinology*, 1937, **21**, 330.

TABLE II.
Effect of Chicken A.P. on Hypophysectomized Rat Ovaries and Adrenals.

Exp.	Treatment	Adrenals, mg.	Uterus, mg.	Ovaries, mg.	Lutein Tissue
A131	90 mg. chick A.P. in 10 days	11.7	107.1	13.6	Traces
	,, ,, ,, ,, ,, ,, ,,	12.6	109.5	38.6	C. lutea
	30 mg. chick A.P. in 4 days	—	66.0	35.5	Traces
	Control	3.8	12.6	2.6	None
	,,	4.5	19.6	1.6	,,
A135	160 mg. chick A.P. in 15 days	3.8	129.8	26.3	C. lutea
	Control	2.8	23.8	2.1	None

oestrous stage and the ovaries contained ripe follicles. Some of the ovarian follicles in one rat had begun to luteinize on the fourth day. In 2 cases, many corpora lutea were formed after prolonged treatment.

The chicken hypophyses were also capable of inducing some repair of the atrophied adrenals. This was indicated by an increase in weight over the controls and marked enlargement of the cortex in every case when examined histologically. No changes were observed in the thyroids.

Evidence has been forwarded that ovulation in the rabbit is due, in part, to the luteinizing hormone.[4] Three adult female rabbits in heat were injected with an aqueous extract equivalent to 40 and 75 mg. of chicken hypophysis. Ovulation occurred in the ovaries of two rabbits receiving the 75 mg. dose but failed with the 40 mg. dose.

Summary. These results indicate that the chicken hypophysis is capable of stimulating both follicular growth and luteinization of the rat ovary. The presence of the luteinizer is verified by 3 different methods which are used to identify the luteinizer in mammalian hypophyses. Witschi, *et al.*,[2] have shown that turkey hypophyses contain the luteinizer, based on the results of corpus luteum formation in the ovaries of 2 hypophysectomized rats. This raises the question as to the function of the luteinizing principle in birds since no corpora lutea form in their ovaries. If the stimulation of ovulation is one of the functions of the luteinizing hormone, its presence in the bird gland may be explained on the basis of this particular function. It is possible that the luteinizing hormone will be found in the anterior pituitary of all vertebrates.

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[4] Foster, M., and Hisaw, F. L., *Anat. Rec.*, 1935, **62**, 75.

9644 P

Effect of Thyroxin on Carbohydrate Metabolism of Hypophysectomized Rats.*

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From the Institute of Experimental Biology, University of California.

Hypophysectomized rats differ from the normal in the disposition of fed carbohydrate, depositing a smaller proportion of absorbed glucose and oxidizing a greater part.[1] However, the actual amounts of carbohydrate oxidized appear to be about the same in the 2 cases; for not only is the oxygen consumption low in hypophysectomized rats, but also the rate of absorption of glucose from the intestine in these animals is decreased by about one-third. Whether, if the intestinal absorption rate were nearer normal, the proportionate disposition of the absorbed carbohydrate would also be different has not been known.

It was found by Althausen[2] that treatment with thyroxin produces a marked increase in the absorption rate of glucose and other sugars in normal rats; therefore, at Dr. Althausen's suggestion (personal communication) the possible effects of thyroxin on the rate of absorption of glucose and its disposition were investigated in hypophysectomized rats.

Hypophysectomized rats, 2 weeks post operative, weighing 150 to 180 gm., were given 10 to 20 gamma of crystalline thyroxin daily for 10 days. Then, after being fasted for 18 hours, the rats were fed glucose and the disposition of the carbohydrate was determined as described elsewhere.[1] Determinations were also made of the carbohydrate levels and respiration of unfed hypophysectomized rats treated with thyroxin.

The amounts of thyroxin given, although very small, were sufficient to increase the glucose absorption rate of the hypophysectomized rats quite to normal. The oxygen consumption was increased about 30%, although being still somewhat subnormal. Under these conditions, the proportionate disposition of the absorbed carbohydrate was practically unchanged from that found in untreated hypo-

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* Aided by grants from the Board of Research of the University of California and the Rockefeller Foundation of New York City.

† Porter Fellow, 1937-38.

[1] Russell, J. A., PROC. SOC. EXP. BIOL. AND MED., 1937, **37**, 31; *Am. J. Physiol.*, in press.

[2] Althausen, T. L., *J. Clin. Invest.*, 1937, **16**, 658.

physectomized rats. The actual amounts of carbohydrate deposited were increased only in proportion to the change in absorption rate; the amount of carbohydrate apparently oxidized was increased approximately in proportion to the increase in oxygen consumption. The maintenance of carbohydrate levels in the fasted hypophysectomized rats was not improved in any case by the thyroxin treatment. It was concluded, therefore, that thyroxin substitution therapy in hypophysectomized rats can completely restore the rate of absorption of glucose from the intestine, as it does the basal metabolic rate; but that it does not otherwise improve the carbohydrate metabolism of these animals.

9645 P

Effects in Female Young Born of Pregnant Rats Injected with Androgens.

James B. Hamilton and William U. Gardner.

From the Department of Anatomy, Yale University School of Medicine.

Hormonal factors are known to influence parturition and the condition of the young. Interference with parturition in the rat has been reported with pituitary and placental extracts and pregnancy urine,[1] pituitary implants,[2, 1e] luteal extracts,[3] hypophysectomy,[4] estrogens,[5] and androgens.[6] The present report deals with some of the permanent effects on female rats whose mothers received androgenic substances during pregnancy.

Study has been made of 10 female young which have grown to maturity and have either been sacrificed or have died from abnormalities resulting from effects of androgen injections in their mothers. Female young, born after adequate androgenic treatment of the pregnant mother (500 gamma of testosterone propionate* in 0.1 cc. of peanut oil injected subcutaneously daily during the last third of pregnancy), superficially resemble males in that the clitoris is large and penis-like, the perineum extensive and scrotum-like, and the pin-point vaginal opening absent.

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[1] *a.* Teel, H. M., *Am. J. Physiol.*, 1926, **79**, 170; *b.* Evans, H. M., and Simpson, M. E., Proc. Soc. Exp. Biol. and Med., 1929, **26**, 595; *c.* Levin, L., Katzman, P. A., and Doisy, E. A., *Endocrinology*, 1931, **15**, 207; *d.* Hoopes, E. C., Proc. Soc. Exp. Biol. and Med., 1934, **31**, 1115; *e.* Hain, A. M., *Quart. J. Exp. Physiol.*, 1932, **22**, 249.

[2] Engle, E. T., and Mermod, C., *Anat. Rec.*, 1928, **38**, 11.

[3] Nelson, W. O., Pfiffner, J. J., and Haterius, H. O., *Am. J. Physiol.*, 1930, **91**, 690.

[4] Pencharz, R. I., and Long, J. A., *Am. J. Anat.*, 1933, **53**, 117.

[5] *a.* Parkes, A. S., *J. Physiol.*, 1930, **69**, 463; *b.* Hain, A. M., *Quart. J. Exp. Physiol.*, 1935, **25**, 131.

[6] *a.* Dantchadoff, V., *Compt. rend. Soc. de biol.*, 1937, **124**, 516; *b.* Greene, R. R., and Ivy, A. C., *Science*, 1937, **86**, 200; *c.* Hamilton, J. B., and Wolfe, J. M., *Anat. Rec.*, in press; *d.* Scipiades, E., Proc. Soc. Exp. Biol. and Med., 1937, **37**, 242.

Outstanding phenomena observed in these animals at maturity were as follows:

(1) Upon gross inspection no vaginal aperture was seen (Fig. 1) and the peripheral portion of the vagina was absent beyond a small tubercle about 1 cm. from the perineum. The fluid-containing cavity within the uterus and cervical portion of the vagina was abruptly terminated at the tubercle.

(2) The nipples were either absent or were markedly underde-

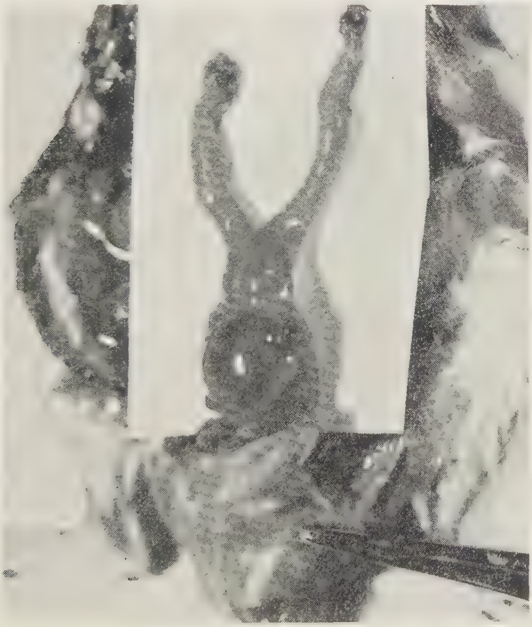

FIG. 1.

Expansion of the cervix and the cervical portion of the vagina by fluid. With successive estrous cycles the swelling may reach dimensions of 35x30x32 mm., and apparently cause death of the animal. Note opposite the pointer the absence of a vaginal introitus.

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* Furnished by the Ciba Company.

veloped. Absence and underdevelopment were seen in the same animal. Mammary glands were nearly normal in appearance.

(3) The internal reproductive system with the above exceptions was intact and some degree of ovarian function was evidenced by uterine, vaginal, and mammary histology, by the presence of follicles and corpora lutea, and by fluid in the uterus and vagina.

(4) The uterus and existent portion of the vagina were greatly bloated with retained fluid in 6 of the animals (Fig. 1). The dimensions of the expanded uterine cervix and vagina were as great as 35x30x32 mm. Apparently this large distension by fluid, which followed the assumption of functional activity of the reproductive system, proved fatal in the majority of these animals, seemingly partly by compression of the colon. The urinary system was also affected, the bladder and urethra being held taut by the subjacent expansion of the reproductive system.

The preliminary study of permanent abnormalities in rats resulting from injections of hormones to the mother during pregnancy, suggest the following inferences:

(a) Pseudohermaphroditism may be produced by androgen injection, the females presenting a masculine appearance with a penis-like clitoris, scrotum-like perineum and an absence of proper development of the nipples and the outer portion of the vagina.

(b) The absence of nipples in the normal male rat may be correlated with the presence of male hormone.

(c) Possible harmful effects on the foetus must be guarded against in clinical use of hormonal substances in large amounts or at critical times during pregnancy.

9646 P

Myenteric Activity Modifications Induced by Caffeine.

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From Long Island University, Brooklyn, New York, and the Marine Biological Laboratory, Woods Hole, Mass.

Enteric motility is dependent largely upon mechanical distension and upon the *chemical* nature of the contents. To study the effect of the purine complex, caffeine, upon the intrinsic myogenic responses[1] of the mammalian small intestine, excised segments of the

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[1] Gasser, H. S., *J. Pharm. and Exp. Therap.*, 1926, **27**, 395.

rabbit intestine were perfused with Tyrode solution separately and with the addition of caffeine. The animals were sacrificed by a blow on the occiput to avoid any possible anesthesia effect. A modification of Trendelenburg's method was used. Comparable segments of the duodenum, jejunum, and ileum were employed under constant conditions of temperature, pressure, perfusion volume rate, time of perfusion, and pH (bicarbonate-buffered).[2, 3]

Normal contractions of the mammalian small intestine occur as rhythmic, long peristaltic beats and in the form of irregular, shorter tonus periodicities. The records were obtained under the natural self-regulatory rhythm of the segment. The excised intestinal segment preparation provides a method for observing the direct response of the musculature to caffeine as absorbed from the lumen through the mucosa. Caffeine-in-Tyrode concentrations varied from 0.05% to 2.0% of the pure alkaloid, U.S.P. Powder (Merck). Magee and Southgate[4] demonstrated differences in the effects of salts upon the several areas of the small intestine. Similarly, in my experiments, the several segments show a different sensitivity to caffeine.

TABLE I.
Effect of 1% Caffeine-in-Tyrode.

	Amplitude	Tonus	Rhythm Rate	Recovery
Duodenum	Decrease (decided)	Increase (decided)	0 or decreased slightly	Complete
Jejunum	Decrease (but less than duodenum)	Increase (but less than duodenum)	0	”
Ileum	Decrease (decided)	Increase (decided)	0	”

Summary. 1. The amplitude and tonus are the factors primarily modified by caffeine. 2. The presence of caffeine in the intestinal lumen in percentages below 0.50% is not effective in general. 3. Effective concentrations tend to increase the tonus and to decrease the amplitude of the contractions. 4. Full recovery occurs except occasionally with the use of caffeine-saturated (approximately) perfusion solutions.

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[2] Cheney, R. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 3.

[3] Cheney, R. H., *J. Pharm. and Exp. Therap.*, 1933, **48**, 470.

[4] Magee, H. E., and Southgate, B. A., *Proc. Physiol. Soc. J. Physiol.*, 1928, **65**, VII.

9647

A Discussion of Some Errors in Quantitative Spectrophotometry.*

Elmer S. Miller. (Introduced by George O. Burr.)

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Recent advances in photoelectric spectrophotometry reported by Hogness, *et al.,*[1] and Smith[2] now permit precise measurements of absorption coefficients. The techniques described by these workers have made it possible for investigators in different laboratories to obtain concordant results. Kuhn,[3] Miller,[4, 5] and Van Der Hulst[6] have pointed out several analytical applications of spectrophotometry in biological chemistry. However, these analytical procedures are valid only when the investigator is cognizant of the limitations imposed by certain errors. This paper concerns anomalous transmission values (or *i. e.,* I_o/I)† that are caused by scattered radiation or absorption due to impurities.

Data presented in Graph 1 illustrate the discrepancies in carotenoid absorption curves of solutions containing 0.6% butter and solutions with equivalent carotenoid concentrations but no butter. In this experiment 20% diethyl ether and 80% ethanol (by volume) were employed as solvent. When the absorption curves were measured on aliquot portions of butter—carotenoid solutions at one hour intervals, the difference between the last curve, in the series, and the standard curve (no butter present) was always less than when similar comparisons were made with curves obtained on earlier runs. When the absorption curves were run on butter solutions that had stood for 15 to 24 hours, no deviation was observed from the standard curves.

In experiment 2, the transmittance was measured on butter-carotenoid solutions in spectral regions where the carotenoids do not absorb. The I_o deflections were adjusted to 100.0 ± 0.5 cm. Table I summarizes the I deflections for butter-carotenoid solutions at

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* Aided by a grant from the Graduate School of the University of Minnesota.

[1] Hogness, T. R., Zscheile, F. P., Jr., and Sidwell, A. E., Jr., *J. Phys. Chem.*, 1937, **41**, 379.

[2] Smith, J. H. C., *J. Am. Chem. Soc.*, 1936, **58**, 247.

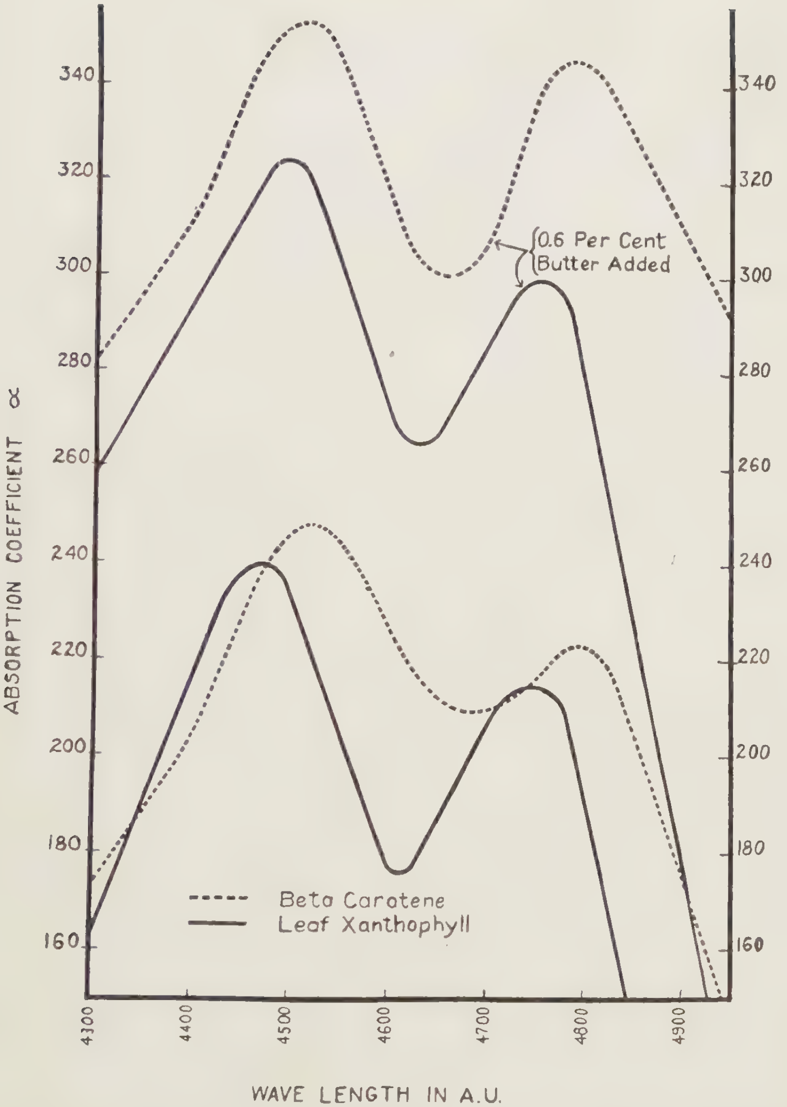
[3] Kuhn, Richard, and Smakula, Alexander, *Z. Physiol. Chem.*, 1931, **197**, 161.

[4] Miller, Elmer S., *J. Am. Chem. Soc.*, 1935, **57**, 347.

[5] Miller, Elmer S., *J. Cereal Chem.*, in press Jan. issue, 1938.

[6] Van Der Hulst, L. J. N., *Rec. Trav. Chim.*, 1935, **54**, 639.

† Same nomenclature as in reference 1.



GRAPH 1.

Absorption spectra of beta carotene and "leaf xanthophyll" in solutions containing 0.6% butter. The lower two curves are included for comparison purposes.

wave lengths 5600 and 6000Å. The deflections (I) for the control cell are recorded in the last column.

Data presented in Table I show that turbidity caused sufficient scattering of light to account for the anomalous absorption coefficients presented in Graph 1.

TABLE I.
Scattered Radiation Due to Turbidity.

Readings made at following intervals Hours	Scattered butter at 5600 Å cm.	Radiation by wavelengths 6000 Å cm.	Scattered radiation in control at $\lambda \left\{ \begin{matrix} 5600 \\ 6000 \end{matrix} \right\}$ Å cm.
Initial	33.9	31.0	—0.4
0.05	33.0	29.0	+0.2
0.2	31.6	27.4	—0.1
1.0	26.4	24.8	+0.2
3.0	25.8	22.0	0.0
16.0	0.8	1.0	+0.5
Solution shaken at end of exp.	35.0	30.6	+ .4

The following data concerns the variations in error in absorption coefficient measurements introduced by impurities in the sample. Table II summarizes the errors in absorption coefficients for beta carotene in the visible and ultraviolet region.[7]

TABLE II.
Errors in Absorption Coefficients for Beta Carotene.

Maxima at wavelengths	Specific α	Maximum deviation ± 3 runs	Error %
4800	222	1.0	0.9
4525	249	1.4	1.1
2790	43	0.5	2.6
Minima at wavelengths			
4700	210	1.0	2.0
3200	10	0.5	10.0
2200	17	1.0	12.0

Hogness, *et al.*,[8] have published the molecular absorption coefficients for ferriheme cyanide (Table III).

Although the data in Tables II and III are for different kinds of substances, the variations of the percent error are in good agreement. From the view point of practical spectrophotometry, it is important to recognize that a 1% impurity on weight basis may introduce a 10% error in absorption coefficients, especially if the coefficient is very small.

Data presented in Graph 1 and Table I show that solutions should be optically clear before accurate transmission measurements are made. If the foreign substance cannot be removed, the amount of

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[7] Miller, Elmer S., *Plant Physiology*, 1937, **12**, 667.

[8] Hogness, T. R., Zscheile, F. P., Jr., and Sidwell, A. E., Jr., *J. Biol. Chem.*, 1937, **118**, 1.

TABLE III.
Absorption Data for Ferriheme Cyanide in a Solution Containing 0.093 M. NaOH and 0.5 M. KCN per liter.

Maxima at wavelengths	Molecular $a \times 10^{-3}$	Maximum deviation ± (2 runs)	Error %
5450	11.0	0.1	1.8
4225	85.5	0.8	1.9
3650	37.5	0.5	2.7
2300	32.1	0.8	5.0
Minima at wavelengths			
5000	7.0	0.7	20.0
3825	33.6	0.3	1.8
3000	12.7	0.2	3.1

scattered radiation due to turbidity can be determined by measuring I (the transmission) in adjacent spectral regions where absorption by the substance investigated is zero. Data in Table I indicate that the error in a after such a correction is made, can be reduced to 2-5%. Under proper conditions, transmission measurements of certain turbid solutions are reproducible, *i. e.,* if readings are made at corresponding time intervals after shaking the solution. When scattered radiation is taken into account, Beer's law is obeyed by carotenoid solutions containing 0.6% butter.

Miller[9] in preliminary attempts to measure the absorption curve for Spirogyra between wavelengths 5000 and 7000Å, found that 2-3% variations insignificant because so much of the absorption was due to scattered radiation.

In spectrophotometry, errors introduced by impurities may be the limiting factor. A simple calculation is presented as an illustration. Many lipids have a specific coefficient (grams per liter) less than 0.5. If the impurity has a specific coefficient of 250, only a 0.02% contamination of the sample will introduce a 10% error in the lipid coefficient.

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[9] Miller, Elmer S., unpublished data.

9648 P

Effects of the Triplo-X Condition on Development in *Drosophila melanogaster*.

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In a study of the giant larva, using an attached-X stock ($\underline{\text{gt bb}^{11}/\text{gt w}^{a}}$), a high pupal mortality was observed, as well as a high frequency of zygotes whose pupation was retarded, as expected for giant, but which did not form large (giant) pupa cases. In order to separate the effects of the triplo-X condition from those of the giant, a study has been made of the larvae and pupae of an attached-X yellow white stock.

In this stock, male larvae and pupae can be identified by the testes, triplo-X females by the ovaries and yellow (wild type) Malpighian tubes, and $\underline{\text{XX}}\text{Y}$ females by ovaries and the colorless Malpighian tubes characteristic of individuals homozygous for white. During this investigation, it was noticed that the homozygous yellow white larvae could be identified by the light color of the mouth armature, a character which could be diagnosed with certainty in the living larvae at all stages from hatching to pupation, and which, when checked against the colorless Malpighian tubes of the homozygous white larvae, proved to be an accurate means of classification. That the light mouth armature color is dependent upon the yellow gene and not the white was determined by examination of white and of yellow stocks. In the first instar larva homozygous for yellow the whole mouth armature is very light brown, almost yellow; wild type armature at this time is very dark brown. In the third instar (about 70 hours until pupation at 25°C.), the mouth hooks are as dark as those of wild type but the posterior part of the armature is light brown. Both Malpighian tube color and mouth armature color were used in classifying larvae in these experiments.

Lethal action of the triplo-X condition. Newly hatched larvae were cultured in 3-inch Petri dishes, 30 to a dish, on finely strained banana-agar; this colorless, translucent medium served as a good background for observation of Malpighian tube color. Of 352 larvae from matings of attached-X ($\underline{\text{y w}}/\text{Y}$) females by wild type males, 47 died before pupation (33 in the first instar, 3 in the second, 11 in the late third), a larval mortality of 13.35 ± 1.81% of the larvae (or 10.01 ± 0.02% of the total zygotes, allowing for death of 25% of the zygotes, Nullo-X, in the egg stage[1]). All dead third instar larvae were identified as triplo-X. It was not possible to ascertain the genotype of all the dead first and second instar larvae, as these small larvae decompose too rapidly after death to allow accurate classification of Malpighian tubes, and the discovery of the difference in mouth armature color was made too late to be used at this juncture. Of 303 pupae, 82 failed to hatch, a mortality of 23.29 ± 2.25% of the larvae, or 17.48 ± 1.75% of the total zygotes; all but 3 of the dead pupae were triplo-X. A few superfemales emerged, with a frequency of 3.13 ± 0.92% of the larvae, or 2.35 ± 0.70% of the total zygotes. The dead larvae and pupae thus constitute 36.64% of the total larval population. The expected proportion of triplo-X individuals is 33.3% of the larvae. It is evident from these data that the majority of triplo-X zygotes die in the pupal stage, while a small proportion die in the third larval instar, and a few in the first and second instars.

Similar counts were then made with the giant stock, in which the high pupal mortality was originally observed; these counts corroborate the evidence from the yellow white stock. Of 298 larvae from matings of attached-X $\underline{\text{gt bb}^{11}}$/Y females by gt w^{a} males, 50 died in the larval stage (41 in the first instar, 1 in the second, 8 in the third), a larval mortality of 16.77 ± 2.16% of the larvae, or 12.58 ± 1.66% of the total zygotes; the pupal mortality was 28.18 ± 2.65% of the larvae, or 21.14 ± 2.04% of the zygotes. The total deaths, in percent of the larvae, were thus 44.95%. Here the chromosomal constitution of the dead individuals could not be ascertained.

Comparison of these data with the mortality characteristic of the Florida wild type stock (about 8% larval, almost entirely in the first instar, and 1% pupal mortality, from unpublished data of the author) lends weight to the conclusion that the lethal action of the triplo-X condition is chiefly exerted upon the pupal stage. Direct comparison of the 3 stocks cannot be made, however, since the genetic backgrounds are different in the 3 cases; the residual mortality in the Florida stock appears to be greater than in the 2 attached-X stocks. It is probable that the processes which lead to death of the triplo-X pupae are begun in the larval stage and result in death of some of the larvae, especially in the stage just before pupation, but that the majority of triplo-X zygotes are able to survive until pupation. A few triplo-X larvae appear to die in the first instar; this is not surprising, since the death of Mw, Ml^2 and Mfla homozygotes and ClB males in the first instar,[2] as well as of almost all of the wild type larvae which die during the larval period, indicates that the first instar is a crucial period in development.

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[1] Li, J.-C., *Genetics*, 1927, **12**, 1; Poulson, D. F., *Proc. Nat. Acad. Sci.*, 1937, **23**, 133.

Delay in onset of pupation. In the y w/+ stock, it was observed that triplo-X larvae do not begin to form puparia until about 24 hours after the mean pupation time of XY and XXY sibs at 25°C. Some *XXX* larvae did not pupate until 7 days after oviposition, and 2 were still in the larval stage at 15 and 18 days respectively. This is the longest delay in onset of pupation reported for any genotype of *D. melanogaster* and is the first delay known to be attributable to a duplication. Dobzhansky[3] has reported a delay in total time of development of superfemales, but the lengthened development has not hitherto been recognized as due to a delay in puparium formation.

Conclusions. The lethal effect of the triplo-X condition is chiefly exerted upon the pupal stage. A few triplo-X zygotes die in the larval period, especially at the stage just before pupation. The onset of pupation of triplo-X larvae is delayed more than 24 hours at 25°C.

The author wishes to thank Professors L. C. Dunn and G. W. Beadle for suggestions, and Dr. C. B. Bridges for advice and help in obtaining stocks.

9649 P

The Estimation of Histidine.

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The estimation of arginine, histidine and lysine in small amounts of protein by the silver precipitation method[1] has been satisfactorily employed by Miller,[2] Mazur,[3] Plimmer,[4] and others. However, Abderhalden and Siebel[5] were only successful in the determination of arginine and lysine. The latter investigators reported large losses of histidine during the purification procedures in which $HgSO_4$ and $CuCO_3$ are employed. It has been recognized that histidine is the most difficult of the basic amino acids to determine accurately[6] and we take this occasion to present some details of the modifications of the 1934 procedure.

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[2] Brehme, K. S., *Genetics*, 1938, **23**, 142; *Am. Nat.*, 1937, **71**, 567.

[3] Dobzhansky, Th., *Biol. Bull.*, 1930, **59**, 128.

[1] Block, R. J., *J. Biol. Chem.*, 1934, **106**, 457.

[2] Miller, E. J., *Biochem. J.*, 1935, **29**, 2344.

[3] Mazur, A., *J. Biol. Chem.*, 1937, **118**, 631.

[4] Plimmer, R. H. A., and Lowndes, J., *Biochem. J.*, 1937, **31**, 1751.

[5] Abderhalden, E., and Siebel, H., *Z. physiol. Chem.*, 1935, **238**, 169.

Histidine is precipitated from the protein hydrolysate at pH 7.4 with $AgNO_3$ and $Ba(OH)_2$.[1] The precipitate is centrifuged, washed and decomposed with H_2S after suspending in dilute H_2SO_4 (*cf.* [7] for apparatus). The Ag_2S is removed and the filtrate and washings are concentrated *in vacuo* (*cf.* [7]). The filtrate is adjusted to pH 3.5-4.5 with $Ba(OH)_2$, and after removal of the $BaSO_4$, the histidine solution is concentrated to approximately 20 cc. and decolorized with charcoal. The precipitate is filtered, thoroughly washed with hot H_2O and the filtrate is diluted to volume (100 cc.). Histidine may be determined directly as follows.

(a) *Colorimetric* (Kapeller-Adler). 2 cc. portions (containing 0.5-1.0 mg. of histidine per cc.) of the sparkling histidine sulfate solution are pipetted into 6 test tubes graduated at 10 cc. Into 6 other similar tubes, an equal quantity of histidine standard is placed. (For best results, standard and unknown should contain approximately the same amounts of histidine.) The 12 tubes are set up alternately and an excess of Br_2 in dilute acetic acid (*cf.* [7]) is added. The bromination is allowed to proceed for 10 minutes, care being observed that all the solutions are colored yellow at the end of this period. The excess Br_2 is removed by a drop of As_2O_3,[8] and 2 cc. of NH_4OH reagent (*cf.* [7]) per tube are added immediately. The contents of the tubes are mixed and they are placed in mildly boiling H_2O for 5 minutes. At the end of this period, the solutions are cooled in ice for 10 minutes and diluted to volume with NH_4OH reagent. The contents of each group of test tubes are mixed and the colors matched in the usual fashion.

(b) *Gravimetric* (nitranilic acid). In 1936, Town[9] claimed that nitranilic acid (2,5-dihydroxy-3,6-dinitroquinone) specifically precipitates glycine from an ethanolic solution of the products of protein hydrolysis. Stein[10] found that the amount of glycine in gelatin estimated by Town's method was greater than that determined by Bergmann and Fox[11] and that this discrepancy could be explained by the partial precipitation of the basic amino acids along with glycine. We have found that nitranilic acid can be used successfully for the isolation of histidine as follows:

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[6] Block, R. J., *J. Biol. Chem.*, 1934, **105**, 455; 1937, **119**, 765.

[7] Block, R. J., *The determination of the Amino Acids*, Burgess Publishing Co., Minneapolis, Minn., 1938.

[8] Conrad, R. M., and Berg, C. P., *J. Biol. Chem.*, 1937, **117**, 351.

[9] Town, B. W., *Biochem. J.*, 1936, **30**, 1833.

[10] Stein, W. H., personal communication.

The remainder of the histidine solution (*cf.* a) is concentrated *in vacuo* (pH 3.5-4.0) and an excess of nitranilic acid (freshly dissolved) in H_2O, $(CH_3)_2CO$, or CH_3OH is added. The finely crystalline, yellow precipitate is filtered off, washed and dried at 110°. The gravimetric and colorimetric estimations check very closely, on analysis of 15 brain proteins, if the factor 0.403 x the weight of the yellow precipitate is employed. Further work along this line is now in progress.

In certain instances, further purification of the histidine fraction is desirable before estimation of the amino acid. In such cases, the histidine sulfate solution is concentrated to approximately 120 cc. and an excess of Denige's reagent (*cf.* [7]) is added. The suspension is centrifuged for 20-30 minutes and the filtrate is discarded. The precipitate is suspended in H_2O and decomposed with H_2S. After removal of the HgS and H_2S, the histidine solution is adjusted to pH 5-6 with $Ba(OH)_2$. $BaSO_4$ is removed and washed. The filtrate is boiled and it is made alkaline to litmus by the addition of minimal quantities of $BaCO_3$. An excess of $CuSO_4$* and a further quantity of $BaCO_3$ to turn red litmus blue are added. The suspension is boiled 2-3 minutes longer, cooled in ice, centrifuged, washed, and after acidification of the filtrate with H_2SO_4, the Cu is removed by H_2S. The solution is concentrated *in vacuo* (pH 3.7-3.9) and the histidine is determined.

Results indicating the probable errors of the method, which are based on more than 10 experiments, each starting with 0.1355 gm. of histidine hydrochloride hydrate are given:

	Loss (%)
Histidine silver precipitate (decolorized)	1-2
" mercury "	4-5
" copper filtrate	2-3
" diflavianate precipitate	15-25
" nitranilate " †	less than 3

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[11] Bergmann, M., and Fox, S. W., *J. Biol. Chem.*, 1935, **109**, 317.

* The use of $CuSO_4$ in place of $CuCO_3$ or $Cu(OH)_2$ was suggested by Dr. Lawrence Morris of Louisiana State University.

† Histidine nitranilate, prepared from histidine hydrochloride hydrate, may be recrystallized from water.

9650

Antigenic Properties of Some Azo Compounds of Serum Albumin and Serum Globulin.

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Mulinos and Schlesinger[1] have shown that when the proteins of egg white or of homologous blood serum are linked to antipyrine through an azo group,* the resulting compounds are antigens capable of eliciting precipitins in rabbits and shock or uterine spasm in guinea pigs. The present report relates to similar experiments with analogous antigens prepared by coupling diazo compounds with homologous serum albumin and globulin.

The results with the azo proteins from serum albumin were analogous to those obtained with whole serum, in that precipitative reactions were readily obtained and were inhibited by the preliminary addition of the original amino compounds employed for the preparation of the azo proteins. On the other hand, the azo derivatives of serum globulin failed to display antigenic character.

The serum albumin and globulins, prepared in the usual manner by precipitation from saturated and half-saturated ammonium sulfate solution respectively, were coupled in weakly alkaline solution, according to the procedure of Landsteiner and Lampl,[2] with the diazo compounds prepared from 4-amino antipyrine and anthranilic acid. After 2 hours at low temperature the mixture was acidified with acetic acid. The flocculent protein was repeatedly redissolved, then reprecipitated by acid, until the supernatant liquor remained colorless. The final azo-protein solutions were made up to contain 10 mg. of protein per cc.

The effects of precipitins from rabbits upon the 4 types of azo protein are shown in Table I; inhibition by amino antipyrine and anthranilic acid is shown in Table II. The cases in which the azo groups showed antigenic reactions may be ascribed to the presence of serum albumin as a contaminant in the original globulin. It is of

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[1] Mulinos and Schlesinger, Proc. Soc. Exp. Biol. and Med., 1937, **35**, 305.

* It was stated in the paper by Mulinos and Schlesinger[1] that antipyrine was diazotized and coupled to protein. In the process the intermediary compound, 4-amino antipyrine, was synthesized and employed.

[2] Landsteiner and Lampl, *Biochem. Z.*, 1918, **86**, 342.

TABLE I.
Precipitative Reactions with Hapten Coupled to Rabbit's Serum Proteins.

Dilution of Antigen	4-amino-antipyrine coupled to: Albumin					4-amino-antipyrine coupled to: Globulin				Anthranilic acid coupled to: Albumin		Anthranilic acid coupled to: Globulin	
	a	b	c	d	e	a	b	c	d	a	b	a	b
1:1000	±	++	+	±	+	±	0	0	0	+	+	±	+
1:2000	++	++++	++	±	+±	++	0	0	0	++±	+±	±	+±
1:4000	+++	++++	++±	+	++	++±	0	0	0	+++	++±	±	+±
1:8000	+++	++±	++±	±	++	+±	0	0	0	+++	++±	±	+
1:16000	+±	++	+±	±	±	+	0	0	0	++	+±	±	±

interest that the azo globulins were approximately twice as intensely colored as the azo albumins.

TABLE II.
Influence of Specific Non-conjugative Chemicals on Precipitative Reactions.

	Antigen			
	A Rabbit's serum albumin coupled to:		B As in A, plus pure	
Animal No.	4-amino antipyrine	Anthranilic acid	Antipyrine	Benzoic acid
65	++++	—	±	—
67	+++	—	±	—
43	++±	—	trace	—
7	+	—	faint trace	—
45	++	—	faint trace	—
41	+±	—	0	—
325	—	+++	—	+
347	—	++±	—	±
827	—	+	—	0
432	—	++	—	faint trace
377	—	++	—	,, ,,

TABLE III
Guinea-pig Uterus: Pigs Injected with Homologous-protein Compounds.

Guinea pig injected with:		No. experiments	Specific spasm of uterus	Specific Inhibition
Anthranilic acid-azo-guinea-pig serum:	Albumin	5	4	3*
	Globulin	5	none	—
4-amino-antipyrine guinea-pig serum:	Albumin	6	4	4†
	Globulin	4	none	—

*By the addition of pure sodium benzoate to the buffered Locke's solution.
†By the addition of pure antipyrine to the buffered Locke's solution.

Table III shows the results of experiments with guinea pigs. The animals were injected with the 4 azo compounds produced from amino antipyrine and anthranilic acid with guinea pig's serum albumin and globulin respectively. After the requisite incubationary period the uteri of the guinea pigs were excised and suspended in warm, oxygenated Ringer's solution, according to the technic of Dale.[3] To one strip of uterus was added the homologous azo protein. If spasm occurred, the strip was washed and another dose of the azo protein was added. If this second addition failed to induce spasm, it was assumed that the first spasm was specific in nature. To a second strip of a uterus found sensitive was added a preliminary dose of the chemical compound corresponding to the azo protein. To a third strip of such a uterus was added another azo protein prepared from a foreign protein (rabbit or egg) and the same amino compound. When a strip of a uterus sensitive to an azo albumin was treated with the simple amino compound, it lost its ability to respond to spasm to the addition of the specific antigen. The strip was therefore rendered insensitive by the amino compounds which were non-antigenic in themselves but had conferred upon the homologous protein antigenic powers for the animal from which it was derived. Of 9 guinea pigs injected with azo globulins derived from anthranilic acid or amino antipyrine, none became sensitive either to the original azo globulin or to the azo albumin. Of 11 animals injected with the azo albumin compounds and studied in parallel with the above, 8 (73%) were positive both to the original azo albumin and also to the azo globulin. Seven of the 8 sensitive uteri showed the phenomenon of "specific inhibition" upon preliminary addition of the corresponding chemical compound; in the eighth uterus the response was present but much diminished. No spasm occurred with the native proteins or with antigens prepared from amino compounds other than those employed in the preparation of the azo protein used for sensitization.

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[3] Dale, *J. Pharm. Exp. Therap.*, 1912, **4**, 167.

Analogous experiments independently undertaken by one of us (DBS) with azo proteins prepared from diazotized sulfanilic acid and rabbit-serum albumin and globulin yielded similar results. The azo proteins were prepared by the method of Heidelberger.[4] The azo albumin was purified by repeated precipitation with acid; the azo globulin was purified similarly but in presence of ammonium sulfate. The purification was continued until the azo proteins gave no precipitate with chicken anti-rabbit serum. Both preparations were redissolved in neutral solution and dialyzed until no more color diffused out. Rabbits were injected with 2.5 mg. of protein 5 times a week for 2 weeks, and 5 mg. of protein 5 times a week for a third week. They were bled on the fifth day after the last injection. Of the 10 rabbits, the 5 injected with the azo globulin were negative, and 3 of the 5 injected with azo albumin were positive. This result was unchanged by a second course of injections. The 3 positive sera gave weak cross-reactions with azo-egg-albumin prepared from diazotized sulfanilic acid. After a third course of injections all of the rabbits injected with azo-albumin compounds gave positive results, while those injected with the azo globulin still gave negative results. Six more rabbits given 2 more courses of injections of azo globulin again gave negative results. The precipitates obtained in the azo-albumin antiazo-albumin reaction were found colorimetrically to contain only about 10% of the colored antigen. In a cross-reaction between azo globulin and antiazo-albumin the precipitate contained less than 4% of the colored component, despite the fact that the azo-globulin solutions are colored more intensely per unit of nitrogenous substance in solution.

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[4] Heidelberger, Kendall, and Soo Hoo, *J. Exp. Med.*, 1933, **58**, 137.

9651 P

Carrion's Disease. I. Behavior of the Etiological Agent Within Cells Growing or Surviving *in vitro*.*

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"Carrion's Disease" commonly includes two very different clinical syndromes—(1) a severe, usually fatal febrile anemia, (Oroya fever) and (2) a remarkable cutaneous eruption of hemangioma-like nodules *(verruga peruana)*. Clinical and experimental evidence strongly suggests that these two conditions are successive stages of a single disease, the first (anemic) stage being of variable severity and often inapparent or even absent.

The generally accepted cause of the first condition (Oroya fever) is a small pleomorphic microörganism, first seen by Barton[1] in or on red blood cells and named *Bartonella bacilliformis*.[2] This organism was grown in Noguchi's leptospira-medium.[3] Apparently identical organisms were also grown from the cutaneous nodules of *verruga peruana;* the organisms cultivated from both conditions produced angiomatous nodules in the eyebrows of monkeys, in which bartonella-like microörganisms were demonstrated histologically.[3, 4] Consequently, notwithstanding certain puzzling discrepancies, this cultivable organism has been almost universally accepted as the cause of both these clinical conditions.

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* This paper, with the four following papers, summarizes certain observations made by the Harvard 1937 Expedition to Peru, organized by Dr. Richard P. Strong of the Department of Tropical Medicine. Other members were Dr. Henry Pinkerton, Department of Pathology, Dr. Marshall Hertig and Dr. David Weinman, Department of Comparative Pathology, and Mr. Byron L. Bennett, Technical Assistant. The work was made possible by the generous coöperation of Dr. Telémaco S. Battistini, Director of the National Institute of Hygiene and Public Health in Lima, and was carried out in this Institute. Assistance rendered by other Peruvian physicians is also gratefully acknowledged. These investigations were aided in part through grants from the Richard P. Strong Fund (founded by the late Dr. Frederick C. Shattuck), the Proctor Fund for the Study of Chronic Disease, and the William W. Wellington Memorial Research Fund.

[1] Barton, A. L., *Cron. Med. Lima*, 1909, **26**, 7.

[2] Strong, Tyzzer, Brues, Sellards, and Gastiaburú, *Report of First Expedition to South America*, Harvard University Press, 1915.

[3] Noguchi, H., and Battistini, T. S., *J. Exp. Med.*, 1926, **43**, 851.

The rickettsiæ of typhus and spotted fever form distinctive and constant patterns within mammalian cells grown *in vitro,* patterns that are identical with those found in infected cells of the arthropod vectors of these diseases.[5, 6] *Bartonella bacilliformis* was found in compact rounded masses in the endothelial cells of lymph nodes, liver, and intestine from fatal cases of Oroya fever.[2] We wished to determine whether the organisms cultivable from Oroya-fever blood and from the cutaneous nodules of *verruga peruana* would behave alike as intracellular parasites when cultivated in association with suitable mammalian cells *in vitro.*

Mesenchymal cells of guinea pig and rat tissues *in vitro* were found susceptible, although these animals have resisted infection. Tissues from the lung, bone-marrow, spleen, and *tunica vaginalis* were used in the plasma-culture[7] and in modified Maitland cultures.[8] Cultures of both types were studied by smears and paraffin sections (Regaud-fixed, Giemsa-stained).

The media were inoculated with one or two drops of material from the following sources: (1) Blood from a severely anemic case of Oroya fever. (2) Cultures from the blood of 7 cases of Oroya fever, showing organisms undoubtedly identical with those described by Noguchi and Battistini, and maintained in leptospira-medium, with bi-weekly transfers, for periods ranging up to 4 months. (3) Similar cultures obtained postmortem from lymph-nodes and bone-marrow of 2 fatal cases of Oroya fever. (4) Similar cultures obtained from blood and from cutaneous nodules from 3 patients with *verruga peruana,* in 2 of whom there had been no apparent fever or anemia. (5) Similar cultures obtained from the blood of monkeys several weeks after inoculation with virus from human Oroya fever and *verruga peruana.*

In tissue-cultures of both types, infected from all of the above 5

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[4] Noguchi, H., *J. Exp. Med.*, 1927, **45**, 175.

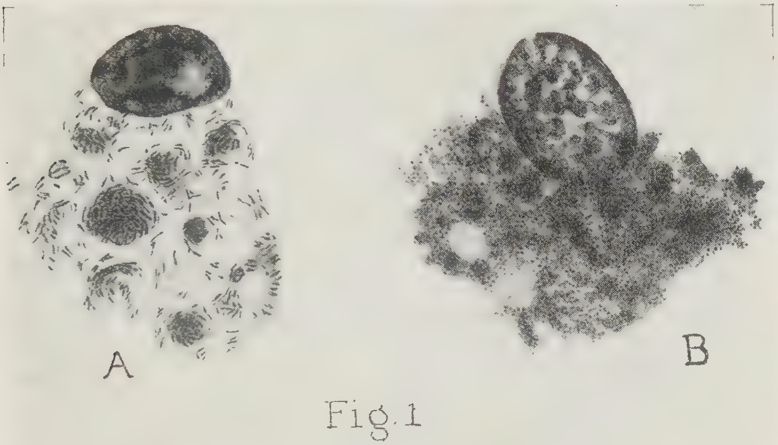
[5] Pinkerton, H., *Parasitology,* 1936, **28** (No. 2), 172.

[6] Hass, G. M., and Pinkerton, H., *J. Exp. Med.*, 1936, **64** (No. 4), 601.

[7] Pinkerton, H., *Arch. f. Exp. Zellforsch.*, 1936, **18**, 343.

[8] Nigg, C., and Landsteiner, K., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 2.

sources, bartonellæ were found in abundance, often growing freely outside of cells, but always multiplying voluminously within the



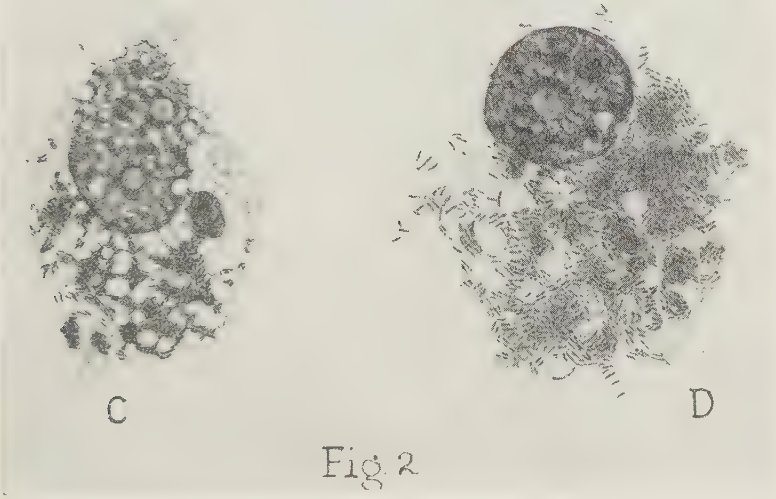


FIG. 1.

Mesenchymal cells from guinea pig's *tunica,* surviving in Maitland medium (Nigg-Landsteiner modification), containing intracytoplasmic clusters of bartonellæ. These cells were infected with cultures of bartonella, obtained in leptospira-medium from the blood of a human case of Oroya fever. Cell A is from a 4-day Maitland culture. Cell B, from an 8-day Maitland culture, shows the granular or globular organisms characteristic of older cultures.

FIG. 2.

Cells infected, under otherwise identical conditions, with cultures of bartonella originating from a human verruga, excised from a patient without fever or anemia. Cell C is from a 5-day culture and cell D is from a 6-day culture.

All cells were drawn from alcohol-fixed Giemsa-stained smear-preparations at a magnification of 1300 diameters.

cytoplasm.† Organisms were absent from controls. In early cultures, small spherical clusters (3-10μ in diameter) of sharply stained diplobacillary rods were seen in the cells. (Fig. 1. Cell A.) Later, usually by the sixth day, many cells were packed with clusters of less discrete organisms, often coccoid, granular, or amorphous, (Fig. 1. Cell B) accurately reproducing the picture seen in human lymphnode-endothelium from fatal cases of Oroya fever. *This characteristic pattern was found in tissue-cultures infected from all 5 sources.*

The mesenchymal cells in which bartonella multiplied were not accurately identified because fibroblasts, endothelial cells, and histiocytes were not distinguishable by the methods employed. In bone-marrow cultures, organisms were absent from granulocytes but often present in megakaryocytes. Erythrocytes, though well preserved in the cultures, were never invaded.

Cell-nuclei were not invaded. The pattern of cell-infection could be easily distinguished from that of other intracellular parasites which we have studied, although individual organisms, when discrete and sharply stained, could easily be mistaken for typhus rickettsiæ.

Conclusions. Organisms cultivated in leptospira-medium, whether from the blood or tissues of Oroya-fever patients or from cutaneous nodules excised from patients with *verruga peruana,* invariably behaved as facultative intracellular parasites when cultivated *in vitro* with growing or surviving mammalian cells. Organisms cultivated from the blood of monkeys infected with human material from either Oroya fever or *verruga peruana* patients behaved similarly. A characteristic and uniform pattern of intracytoplasmic clustering was obtained with cultures from all of the above sources, this pattern being essentially identical with that seen in naturally infected human cells in both conditions (see Paper II). These observations substantiate the belief that Oroya fever and *verruga peruana* are caused by the same microörganism.

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† In their behavior under these conditions, these organisms resemble *B. tularense* (unpublished observations) and differ from typhus and spotted fever rickettsiæ, which grow only within cells. The resistance of these rickettsiæ to cultivation in cell-free media is probably correlated with this behavior.

9652 P

Carrion's Disease. II. Comparative Morphology of the Etiological Agent in Oroya Fever and *Verruga Peruana*.

HENRY PINKERTON AND DAVID WEINMAN.

From the Departments of Pathology and Comparative Pathology, Harvard Medical School, Boston.

The material for this study included human tissues removed postmortem from 3 fatal cases of Oroya fever, and cutaneous nodules excised from 7 cases of *verruga peruana,* 2 of which were apparently afebrile and non-anemic. Cutaneous nodules, produced in monkeys with material from both Oroya fever and *verruga peruana* were also excised for comparative histological study. Impression-smears and paraffin sections were prepared from all of the above types of tissue. Air dried impression smears were stained by the Giemsa method with or without previous fixation in absolute methyl alcohol. For the demonstration of bartonellæ in sections, a technic involving the combination of Regaud-fixation and Giemsa-staining was found to be invaluable. This combination was used for the demonstration of *Rickettsia ruminantium* by Cowdry[1] and for the demonstration of bartonellæ in verrugas by Noguchi and Battistini.[2] The technic used in this work was that evolved by one of us[3] for staining typhus and spotted fever rickettsiæ.

Oroya Fever. In 2 of the 3 fatal cases of Oroya fever* in which postmortems were performed, the endothelial cells lining blood and lymph channels of many organs were distended with clusters of organisms, as previously described[4] (Fig. 1, Cell A). In many cells these organisms were discrete and rod-shaped but frequently they were represented by granular or amorphous spheroidal masses, recognizable only by their characteristic staining reaction. This picture was found in lymph nodes, liver, (Kupffer cells), bone marrow, spleen, kidney, adrenal, pancreas, thyroid, and testicle. Infected and distended cells were present in all these organs in such numbers that they were prominent on low-power examination. Occasional infected cells were found in the heart and lungs. The distribution of infected cells suggested that they were largely members of the reticulo-endothelial system. In the Kupffer cells of the liver, less than 20% of the infected cells showed the characteristic clustering of organisms, the remainder being uniformly filled with bartonellæ. In all other organs, more than 90% of the infected cells showed the characteristic clustering.

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[1] Cowdry, E. V., *J. Exp. Med.,* 1925, **42**, 253.

[2] Noguchi, H., and Battistini, T. S., *J. Exp. Med.,* 1926, **43**, 851.

[3] Pinkerton, H., *J. Exp. Med.,* 1931, **54**, 181.

* The third case was complicated by a secondary infection and relatively few bartonellæ were found in the erythrocytes at the time of death.

[4] Strong, Tyzzer, Brues, Sellards, and Gastiaburu, *Report of First Expedition to South America,* Harvard University Press, 1915.

We are inclined to interpret the various appearances of the organisms, ranging from discrete bacilli to granular and even homogeneous masses as pleomorphism rather than as stages in a progressive life-cycle. Even in leptospira-medium, the organisms go through similar changes.

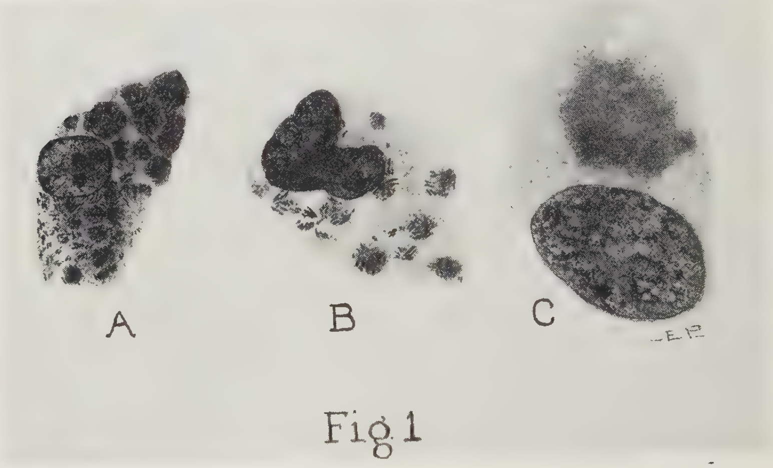

FIG. 1.

Cell A. Kupffer-cell in liver from a fatal case of human Oroya fever, showing intracytoplasmic clusters of bartonellæ. From a Regaud-fixed, Giemsa-stained paraffin section. × 1300.

Cell B. Endothelial cell from a human cutaneous nodule excised from a patient with afebrile and non-anemic *verruga peruana*. Note similarity of intracytoplasmic bartonellæ, both morphologically and in their distribution to those seen in Oroya fever (Cell A). From an alcohol-fixed Giemsa-stained smear preparation. × 1300.

Cell C. Endothelial cell from the same preparation as Cell B, showing the more homogeneous, bright-red-staining type of inclusion. Intermediate stages were found between this picture and that shown in cell B. × 1300.

Verruga peruana. In 10 cutaneous nodules (representing 7 different patients) bartonella-filled cells were invariably present, often in enormous numbers. Distention of cells was much less common than in the organs of Oroya-fever cases. Clustering of organisms in cells was rarely as pronounced as in Oroya-fever organs, but was often definite. (Fig. 1, Cell B.) In smear-preparations, organisms appeared to be entirely intracellular in position. In paraffin sections, no definite evidence of extracellular multiplication was seen, although the presence of bartonellæ in elongated cytoplasmic processes of spindle-cells made it difficult to determine this point. In several verrugas, particularly in those containing fewer cells definitely filled with bartonellæ, red-staining "inclusions" occupying from 10 to 75% of the cytoplasm were seen (Fig. 1, Cell C). These structures, sharply demarcated from the uninvolved blue-staining cytoplasm, were finely granular and most often approximately spherical. They are undoubtedly the structures described by Mayer, Rocha Lima, and Werner,[5] and considered by them as similar to "chlamydozoa". Intermediate forms between these structures and definite clusters of bartonellæ were seen. Whether they represent clusters of degenerating organisms or a stage in a life-cycle has not been ascertained. They have, in many instances, considerable resemblance to clusters of the psittacosis organisms as described by Bedson and Bland[6] and by Bland and Canti.[7]

Cutaneous Nodules from Monkeys. No differences were observed between the cutaneous nodules (verrugas) produced in the eyebrows of monkeys by the injection of emulsified human Oroya-fever lymphnodes and those produced by injecting emulsified cutaneous nodules from cases of *verruga peruana.* Such lesions invariably contained numerous bartonella-filled cells identical with those seen in human verrugas and frequently also contained red-staining "inclusions" of the type described above.

Conclusions. Bartonella-filled cells were found in large numbers in nearly all organs of human Oroya fever cases, in cutaneous nodules from afebrile and non-anemic cases of *verruga peruana,* and in cutaneous nodules produced in monkeys by inoculation with human material from both of the above sources. The morphological identity of the organisms, and the essential identity of the patterns produced by them within cells in all of the above tissues furnish additional evidence that Oroya fever and *verruga peruana* have a common etiological agent, *Bartonella bacilliformis.*

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[5] Mayer, M., Rocha Lima, H., and Werner, H., *Munch. Med. Woch.*, 1913, **60**, 739.

[6] Bedson, S. P., and Bland, J. O. W., *Brit. J. Exp. Path.*, 1932, **13**, 461.

[7] Bland, J. O. W., and Canti, R. G., *J. Path. and Bact.*, 1935, **40**, 231.

9653 P

Carrion's Disease. III. Experimental Production in Animals.

David Weinman and Henry Pinkerton.

From the Departments of Comparative Pathology and Pathology, Medical School, Harvard University, Boston.

Considerable experimental evidence supports the etiological unity of *verruga peruana* and Oroya fever. In paper IV of this series, a third form of human bartonella infection—the asymptomatic form, detectable only by blood culture—is described. If Oroya fever and *verruga peruana* are caused by a single agent, then virus from either should under suitable conditions, produce both forms of the disease in susceptible animals. The cutaneous form *(verruga peruana)* has been produced in monkeys, directly by the inoculation of verruga nodules[1] and indirectly by the inoculation of cultures of *Bartonella bacilliformis* derived from either form of the disease.[2, 3] The direct inoculation of human Oroya fever material, however, has never, in the past, produced verrugas in monkeys, and the few instances of apparent reproduction of the anemic form (Oroya fever) are not entirely convincing since the possibility of preëxisting bartonella infection was not eliminated. Spontaneous infections of the monkey with Bartonella and related organisms have been reported[4, 5, 6] and the errors which may arise from the use of such animals have been emphasized by Weinman.[7]

Verrugas, identified histologically and by the demonstration of bartonellæ, were produced by the authors in 4 *M. rhesus* monkeys. Lesions developed only in the supra-orbital regions, at the sites of inoculation. In 2 instances the inoculum originated from *verruga peruana* patients (in one case from verruga-tissue; in the other from a positive blood culture). In the other 2 instances, the inoculum was an emulsion of lymph nodes containing many bartonella-filled cells, removed postmortem from a fatal case of Oroya fever. Thus, we were able to produce the cutaneous form of the disease in monkeys by the inoculation of human material both from the cutaneous and from the anemic forms.

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[1] Jadassohn, J., and Seiffert, G., *Z. Hyg. u. Infek.*, 1910, **66**, 247.

[2] Noguchi, H., and Battistini, T. S., *J. Exp. Med.*, 1926, **43**, 851.

[3] Noguchi, H., *J. Exp. Med.*, 1927, **45**, 175.

[4] Pessoa, S. B., and Prado, A., *Rev. Biol. e. Hyg.*, 1927, **1**, 116.

[5] Leger, A., *Bull. Soc. Path. Exot.*, 1922, **15**, 679.

[6] Malamos, B., *Zbl. f. Bakt.*—I, 1935, **134**, 152.

[7] Weinman, D., *Les Parasites Érythrocytaires Révélés par la Splenectomie: Bartonella et Epérythrozoon,* Paris, Amédée Legrand, 1935.

The anemic form of the disease was reproduced in one monkey, splenectomized 2 months prior to inoculation, and showing no bartonellæ in blood films during this period of observation. Two cc. of blood from a fatal human case of Oroya fever was injected intravenously and a small amount into the inguinal lymphnodes. Death occurred 26 days after inoculation and was preceded by a severe progressive anemia.

At death, 7% of the red blood corpuscles were infected with bartonellæ, each infected cell presenting one to 12 organisms. Morphologically, these bartonellæ appeared identical with those seen in infected human erythrocytes. The blood of this animal yielded characteristic cultures. Necropsy showed pulmonary tuberculosis and hematophagous nematodes in the intestine, infections which may have played a part in the production of the anemia. The organisms in the erythrocytes, however, were undoubtedly *Bartonella bacilliformis* of human origin.

Finally, in 3 non-splenectomized monkeys, the asymptomatic form of the infection was produced. Blood from a fatal case of Oroya fever was injected intravenously in the first monkey, intravenously and into a lymphnode in the second, and intracutaneously in the third. No clinical evidence of the disease developed, but blood cultures were positive for *Bartonella bacilliformis.*

Bartonella muris-free rats, splenectomized or normal, newly weaned and splenectomized adult rabbits, and chickens were not found susceptible to any form of Carrion's disease.

The interpretation of the results of inoculating Peruvian guinea pigs was complicated by the discovery of an undescribed spontaneous bartonella infection in these animals. This problem will be discussed in paper IV.

Summary. The 3 types of infection seen in human Carrion's disease have been reproduced in the monkey. The cutaneous form *(verruga peruana)* was produced in monkeys by the inoculation of (a) infected lymph nodes from a patient with the anemic form (Oroya fever), or (b) human verruga tissue, or (c) cultures of *Bartonella bacilliformis* from the blood of human verruga cases. The anemic form (Oroya fever) was reproduced in one splenectomized monkey, free from spontaneous bartonella infection, by the inoculation of blood from an Oroya-fever patient. This animal died showing a multiple infection of his red cells with *Bartonella bacilliformis.* The asymptomatic form was produced in monkeys by the inoculation of blood from Oroya-fever patients.

9654 P

Carrion's Disease. IV. Natural Sources of Bartonella in the Endemic Zone.

David Weinman and Henry Pinkerton.

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This paper presents the results of a somewhat limited study of the possible reservoirs of the virus of Carrion's disease (Oroya fever and *verruga peruana*). The investigations were, for the most part, carried on in Peruvian villages in the zone of endemicity of the disease. The methods consisted chiefly of the examination of blood films and the making of blood cultures in leptospira-medium in an effort to detect *Bartonella bacilliformis* in human and animal inhabitants of the verruga zone. At Callahuanca, a village in the endemic district, blood cultures and films were obtained from a random group of 53 residents. None of this group gave any sign or symptom of Carrion's disease. Positive blood cultures of *Bartonella bacilliformis* were obtained, however, from 5 of these persons. In none of the 5 could bartonellæ be demonstrated in blood films. Three cases had no past history of Carrion's disease; the other 2 gave a history of *verruga peruana* 6 months previously.

These asymptomatic cases are not hospitalized and continue to reside in the verruga zone where they live in close proximity to the presumptive vector, and inhabit dwellings which render them easily accessible to hematophagous arthropods. It is, therefore, possible that they may constitute an important reservoir of virus for Carrion's disease.

The only animals from the verruga zone available in sufficient numbers to permit of study were guinea pigs and dogs. One guinea pig coming from San Bartolomé, a village within the region where Carrion's disease is endemic, was found to be spontaneously infected with an organism of the bartonella group. Further investigation showed that the laboratory stock of guinea pigs (all of Peruvian origin) was likewise infected. No indubitable spontaneous bartonella infection has been previously reported in splenectomized guinea pigs studied in various countries.[1, 2, 3] We have not found such infection in guinea pigs obtained in Boston.

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[1] Schwetz, J., *Bull. Soc. Path. Exot.*, 1934, **27**, 515.

[2] Schin, C., *J. Chosen Med. Assn.*, 1930, **20**, 1073.

[3] Noguchi, H., *J. Exp. Med.*, 1928, **47**, 235.

Four animals, or about 17%, of a series of 23 splenectomized Peruvian guinea pigs were found to be infected. The first animal (origin unknown) had been inoculated with human Oroya fever blood one day prior to splenectomy. The other 3 animals were not inoculated; one came from San Bartolomé, while the other 2 were of unknown origin. The parasite was observed only after removal of the spleen. The period of incubation after splenectomy varied from 5 to 9 days. The parasites were observed in or on red cells, of which about 0.1% were infected. A single erythrocyte presented from 8 to 15 microörganisms, staining an intense, slightly violet red with Giemsa's solution. The parasites were rod-shaped, and frequently took an accentuated bipolar stain. Their average measurements were 2μ in length by ½μ in width, with many longer and shorter forms.

An attempt was made to cultivate this guinea pig bartonella. Several tubes of leptospira medium were inoculated with the blood of the naturally infected guinea pig from San Bartolomé. In these tubes, kept at 28°C., structures resembling clumps of *Bartonella bacilliformis* had developed by the tenth day. These cultures were injected, 2 months later, into 5 bartonella-free splenectomized guinea pigs obtained in Boston. After 2 weeks, 4 of these inoculated animals presented in their blood films the same organism seen in the naturally infected animals in Peru. This guinea pig bartonella has not yet been fully identified, but evidence at present available indicates that it is not *Bartonella bacilliformis.*

Fifteen non-splenectomized Peruvian guinea pigs inoculated with either infected blood, verruga tissue, or cultures of *Bartonella bacilliformis* did not develop any symptoms of Carrion's disease, nor were bartonellæ found in the blood films.

Verruga peruana is reported to occur in dogs. We observed neither cutaneous lesions nor parasites in the blood, and obtained no positive blood cultures from 10 dogs, of which 4 came from Puente Verrugas, the other 6 from Callahuanca. The dog bartonella, *B. canis,* of wide geographic distribution, has been found in Peru.[4]

Monkeys, which are receptive to the disease, are not reported to occur in the verruga zone. They, therefore, cannot play any important rôle as a natural reservoir of virus.

Summary. Healthy inhabitants of a village in the verruga zone were discovered to be asymptomatic carriers of *Bartonella bacilliformis.* Five individuals of a group of 53 investigated yielded positive blood cultures. Three of these had no past history of Carrion's disease; 2 had previously suffered from *verruga peruana.* It is possible that asymptomatic cases may constitute a reservoir of virus which is important in the natural transmission of the disease.

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[4] Weiss, P., and Muzzo, J. P., *Actualidad Med. Per.*, 1936, **2**, 330.

Peruvian guinea pigs were found to be spontaneously infected with an unidentified bartonella. This infection has not been found in guinea pigs from other regions than Peru, but was transmitted to splenectomized previously uninfected stock guinea pigs obtained in Boston. This guinea pig bartonella is being further studied, in an attempt to identify it more accurately.

9655 P

Carrion's Disease. V. Studies on Phlebotomus as the Possible Vector.

MARSHALL HERTIG. (Introduced by Henry Pinkerton.)

From the Department of Comparative Pathology, Medical School, and School of Public Health, Harvard University, Boston.

Shannon[1] collected Phlebotomus sandflies in the verruga zone of the Rimac Valley, Peru, and shipped them under refrigeration to New York where they were injected into monkeys.[2] Four of 10 batches, including chiefly *Phlebotomus noguchii* and *P. verrucarum* produced inapparent infection with *Bartonella bacilliformis* as evidenced by blood culture. Microscopic examination of these sandflies was not reported. These experiments demonstrated the harboring but not the actual transmission, of bartonella by Phlebotomus, since no living sandflies were used.

During March to June, 1937, the writer collected sandflies in Verrugas Canyon where much of Shannon's field work was done. Houses yielded almost entirely *P. verrucarum;* material from excavations included both *P. noguchii* and *P. verrucarum.* They were collected and transported in 10x75 mm. cotton-stoppered test tubes, inverted in moist pots. Moist plaster-lined pots were used for rearing.[3] Larvae were fed on moist guinea pig's feces. A second method was devised, permitting more ready examination of larvae and more suitable for rearing progeny of individual females. Gravid females, kept at high humidity in their original tubes, laid eggs chiefly on cotton stoppers. The egg-bearing portion was then transferred to a small test tube, completely lined with plaster of Paris, except for a long, slit-like window. This tube was then stoppered with cotton and inverted in a moist pot.

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[1] Shannon, R. C., *Am. J. Hyg.*, 1929, **10**, 78.

[2] Noguchi, H., Shannon, R. C., Tilden, E. B., and Tyler, J. P., *J. Exp. Med.*, 1929, **49**, 993.

[3] Young, C. W., and Hertig, M., PROC. SOC. EXP. BIOL. AND MED., 1926, **23**, 611.

Laboratory-reared adults were not available for experimental work, since both species required about 3 months from adult to adult at 20-23°C. Wild sandflies of both species, but chiefly *P. verrucarum,* were therefore used exclusively in the following experiments.

Examination of Wild Sandflies. Giemsa-stained smears of 15 wild sandflies revealed no bartonella-like organisms. Preliminary examination of Regaud-Giemsa sections has shown minute extracellular cocco-bacillary organisms forming an irregular layer over the entire surface of the mid-intestinal epithelium of one wild female *(P. verrucarum).* Intestines of 26 wild female sandflies yielded no growth in leptospira medium in 23 cases; contaminants in 3.

Sandflies Fed on Patients. An organism morphologically consistent with *Bartonella bacilliformis* appeared in the intestinal contents of about 75% of 90 wild sandflies after feeding on 4 patients showing many bartonellæ in blood films. Sandflies fed on these same patients after the bartonellæ had disappeared from the blood stream, or on other patients with negative blood films at the time of feeding were invariably negative. These organisms were found in large numbers in the brown fecal drops passed 4-5 days after feeding; they were practically absent from clear or whitish fecal drops. They were absent from the feces of wild sandflies fed in nature. In sections of sandflies they appeared in large numbers, occasionally in dense spheroid colonies and usually in the lumen of the mid-intestine. They were never intracellular in any organ. In one instance they were found around the proventricular fold, extending through the short oesophagus to its opening into the pharynx, but they were not found elsewhere in the pharynx. These organisms failed to grow in leptospira medium and their pathogenicity has not been tested; their identity, therefore, remains uncertain.

Transmission Experiments. A series of wild sandflies fed on patients were given opportunity to refeed on 2 rhesus monkeys. Sandflies fed in the laboratory refeed with reluctance. One monkey was bitten by only 2 flies out of 49; the other by 5 out of 50. One monkey died within 4 weeks under circumstances which prevented examination. The other yielded negative blood cultures up to 14 weeks.

Over a period of several weeks, recently captured sandflies were applied to the belly and eyebrows of 2 monkeys. One was bitten by 70 out of 242, and the other by 84 out of 335. Blood cultures up to 4 months were negative.

Two monkeys were inoculated intraäbdominally and endermally on the eyebrow, with suspensions of 10 batches totalling 340 wild females and 5 males. One monkey died within 20 days and was negative. The other received an additional suspension of 70 females and yielded only negative blood cultures up to 4 months.

Summary. Sandflies *(P. verrucarum* and *P. noguchii)* fed on patients showing many bartonellæ in blood films became infected with a bartonella-like organism not as yet identified. A similar infection was found in only one of the wild sandflies examined.

Transmission experiments with sandflies and rhesus monkeys resulted negatively, but were too limited to be conclusive.

9656

A Color Test for Pentoses.

HENRY TAUBER.

From the Research Laboratories of the McLeod Infirmary, Florence, S. C.

One drop (0.05 cc.) of a pentose solution (l-arabinose, d-arabinose, xylose or ribose) containing 0.05 mg. or more of the sugar is placed in a test tube. 0.5 cc. of benzidine solution (1 gm. benzidine in 25 cc. glacial acetic acid. This solution keeps for 4 days) is added and the mixture is brought to vigorous boiling. The test tube is then put in cold water. Within a few seconds a very stable cherry red color develops. Glucose, fructose and galactose give yellow to brown colors with the benzidine solution. Even large amounts of hexoses, however, do not interfere with the test. There is only a slight delay in the formation of the red color (0.1 mg. of arabinose and one mg. of glucose). As little as 10 gamma of pentose may be detected. For instance urine specimens containing 0.1 mg. of arabinose per cc. gave a distinct red color when 0.1 cc. of urine was added to 0.5 cc. of benzidine solution. Normal and abnormal urinary constituents do not interfere with the test. Too large amounts of proteins may be removed from pathological urines by adding an equal volume of 10% trichlor acetic acid, mixing, and heating to 95°. The filtered urine may then be tested.

When testing for pentose in urine, the urine (0.1 cc.) and benzidine solution (0.5 cc.) is brought to vigorous boiling. The mixture is cooled under tap water and 1 cc. of distilled water is added. In the presence of pentose a pink to red color is shown immediately, whereas if pentose is absent the mixture has a yellowish brown color.

This color test is also given by vitamin B_2 (riboflavin), by the yellow oxidation enzyme of Warburg and Christian, and by nucleic acids owing to the ribose content of all of these compounds. It is negative, however, with gum arabic since this pentosan is not hydrolyzed by the benzidine-acetic acid solution. The test is highly specific for pentoses.

9657 P

Direct Effect of Adrenal Cortical Hormone upon Blood Pressure in Shock Induced by Intestinal Manipulation.

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The writers[1] have repeatedly called attention to the slow but steady decline in blood pressure which occurs in adrenalectomized dogs after withdrawal of cortical hormone, and have pointed out that following administration of hormone to animals prostrate from adrenal insufficiency the rise in arterial pressure from shock levels to normal is one of the first and most dramatic changes observed during recovery. The fall in pressure is not a terminal event but is one of the earliest changes from normal exhibited by the animal not receiving hormone.

During the past year we have performed 4 different types of experiments on adrenalectomized dogs, the results of which show that the cortical hormone exerts a direct effect upon blood pressure which is distinct and separable from the action of this hormone upon blood volume, and fluid and electrolyte distribution. Table I gives the essential data obtained from a representative case in one type of experiment in which intestinal stripping was used to induce shock. Dogs lacking adrenals do not spontaneously raise their lowered blood pressure or recover from this treatment.[2]

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[1] Swingle, W. W., Pfiffner, J. J., Vars, H. M., Bott, P. A., and Parkins, W. M., *Science*, 1933, **77**, 58; *Am. J. Physiol.*, 1934, **108**, 428.

Seven active, vigorous, adrenalectomized dogs kept for one year in the laboratory on maintenance doses of cortical hormone, were used. The hormone was withheld 18 hours previous to use in the experiments. The animals were in normal health, at peak weight, and revealed no deviations from normal in their blood and urine chemistry, water balance and arterial pressure. They were etherized and a portion of the small intestine vigorously stripped through the fingers for 20-30 minutes. The dogs recovered rapidly from the anesthetic and their activity and vigor appeared undiminished. However, within the ensuing 6-12 hours the arterial pressure declined to shock levels—40-50 mm. Hg. When the dogs were at the point of collapse they were injected intravenously with 3 cc. per kg. body weight of hormone. The arterial pressure slowly but steadily rose and attained the normal level within 36-48 hours. All shock symptoms disappeared and activity and vigor returned. Arterial pressure increases of as much as 5-10 mm. Hg. per hour were not infrequently observed during the first hours following hormone injections.

Study of the serum Na, Cl and K showed negligible changes in concentration of these electrolytes. In general the Na and Cl tended to increase slightly and the K to fall. About one-half of the dogs did not show hemoconcentration or loss of extracellular fluids, when in profound shock. Three animals exhibited moderate concentration of the blood in the terminal stages, but in no case was disturbance of fluid or electrolyte balance adequate to account for the vascular collapse.

Hypertonic saline (20 cc. of a 20% solution intravenously) also restored the arterial pressure of these animals to normal but the effect was temporary compared to the lasting effect of hormone.

We do not know the locus of action of cortical hormone upon blood pressure. However, it may not necessarily be through the mediation of the nerve endings but may be directly upon the musculature of the vascular system. This point is now under investigation. Overdosage effects, either in intact or adrenalectomized dogs, have not been observed despite use of massive doses of hormone.

Hoskins and Fierman[3] have described a prolonged pressor effect

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[2] Swingle, W. W., and Parkins, W. M., *Am. J. Physiol.*, 1935, **111**, 426.

[3] Hoskins, R. G., and Fierman, J. H., *Endocrinol.*, 1937, **21**, 119.

TABLE I.
Direct Effect of Cortical Hormone on Blood Pressure.

Date	Time, hours	Blood pressure, mm. Hg.	Pulse, min.	Serum Sodium per liter, m.-eq.	Serum Chloride per liter, m.-eq.	Serum Potassium per liter, m.-eq.	Hemoglobin, gm. %	Red cell volume, %	R.B.C., mil.	Remarks
9/23/37	9:00 a.m.	101	84	141.6	109.8	5.9	10.8	35.1	6.24	Normal
,,	10:20-43									Intestine stripped
,,	11:00 a.m.	110	192							
,,	2:20 p.m.	88	176							Inactive
,,	4:20 ,,	67	160							Listless, lethargic
,,	6:00 ,,	42	148	145.0	112.6	5.5	11.3	38.0	6.62	Shock, extract*
9/24	10:00 a.m.	84	112							Improvement
,,	10:00 p.m.	92	100							Strong, active
9/25	11:00 a.m.	106	88	145.5	115.2	5.4	10.5	35.3	5.31	Normal

*3 cc. per kg. extract injected intravenously at 6:00 p.m. 9/23, 1.5 cc./kg. intraperitoneally at 11:00 p.m.—1.5 cc./kg. intraperitoneally at 10:00 a.m. and at 10:00 p.m. on 9/24/37. Food and water withheld during course of this experiment.

following oral administration of glycerine extracts of adrenal cortex to human patients. Recent work on experimental hypertension indicates that the cortical hormone is concerned with blood pressure.[4, 5]

The observation that cortical hormone has a direct effect upon blood pressure of adrenalectomized dogs which is separable and distinct from its functional control of the internal fluid and electrolyte balance of the body, in no way invalidates the earlier conclusions based upon the hormone's "salt-water" action, as offering a rational explanation of many of the phenomena occurring in adrenal insufficiency. The 2 effects of the hormone when considered together, adequately explain the circulatory collapse of adrenal insufficiency.

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Agar Slant Tissue Cultures of Typhus Rickettsiæ (Both Types).

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For some time the writers have been engaged in efforts to obtain typhus fever Rickettsiæ cultures on a scale sufficiently large to permit vaccine-production. While the X-rayed-rat technic, elsewhere published by one of the writers with Castaneda,[1] is entirely adequate for vaccine-production with the murine strains, it has consistently failed with the classical European type. Tissue-culture vaccine-production would undoubtedly be the most practical method of producing vaccines on a large scale against the European disease, and the effectiveness for immunization of formalin-killed tissue-culture organisms has already been demonstrated, both by Kligler and Aschner[2] and by one of the writers with Macchiavello.[3]

Work done on the physiology of Maitland cultures by one of the writers with Schoenbach[4] indicated that whereas virus agents multiply in such cultures only during the period of active tissue metabolism—the Rickettsiæ begin to multiply most profusely after metabolism has slowed down or ceased.

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[4] Goldblatt, H., *Ann. Int. Med.*, 1937, **11**, 69.

[5] Jeffers, W. A., Lindauer, M. A., and Lukens, F. D. W., Proc. Soc. Exp. Biol. and Med., 1937, **37**, 260.

[1] Zinsser, H., and Castaneda, M. R., Proc. Soc. Exp. Biol. and Med., 1932, **29**, 840.

[2] Kligler, I. J., and Aschner, M., *Brit. J. Exp. Path.*, 1934, **15**, 337.

[3] Zinsser, H., and Macchiavello, A., *J. Exp. Med.*, 1936, **64**, 673.

[4] Zinsser, H., and Schoenbach, E. B., *J. Exp. Med.*, 1937, **66**, 207.

This encouraged the hope that it might be possible to develop a method of Rickettsia multiplication in which larger amounts of tissue—and consequently a larger yield of the organisms—could be used. The problem was to find a technic in which the tissue could be held to a minimum of metabolic activity under circumstances of controlled pH and retardation of autolytic change.

Along these lines of effort we have been able to obtain large yields of Rickettsiæ of both the murine and classical (European) types by the following method:

A 4% agar solution in water is mixed with an equal volume of double strength Tyrode's solution to which 50% horse serum has been added before filtration. Phenol red is added in order that the reaction of the tubes may be observed. The initial reaction should be approximately pH 7.4 to 7.6.

Guinea pig tunica is minced, as for Maitland culture, and is heavily inoculated by dropping the centrifugalized concentrate of a preceding culture on the tissue in a Petri dish. After 5 or 10 minutes at room temperature, this tissue is then "buttered" on the surface of the agar, and the tubes are closed with a rubber stopper through which there has been passed a glass tube drawn to a capillary, as shown in Fig. 1. The purpose of the glass tube is that by either breaking or sealing the capillary and allowing any formed carbon dioxide to escape or to accumulate, the reaction can be to some extent controlled, if that becomes necessary. The most important single point in the technic essential to success is reaction-adjustment, and it seems that in most cases, if the initial reaction is as stated, adjustment by the capillary is not necessary in the course of the 8 or 10 days necessary for development of the cultures.

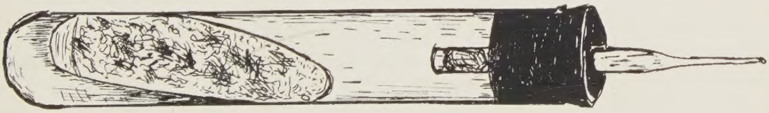

Fig. 1.

At the end of 6 days, multiplication is already apparent and after 10 days smears from the tissue on the surface of the agar show growth of Rickettsiæ. The organisms not only crowd many of the cells which are reasonably preserved, but considerable numbers of scattered Rickettsiæ are found throughout the tissue detritus.

Whether or not these actually grow in the diffused cell material or represent the yield of ruptured cells has not yet been determined. At any rate, a single culture of this kind gives a better total yield than three or four of the larger Maitland flasks.

It is still to be determined why large amounts of tissue on slanted agar do not inhibit Rickettsial growth in the same way in which even a slight excess of tissue prevents multiplication in liquid cultures, even when reaction-changes are controlled. It is probable that since active metabolism seems to delay rather than favor Rickettsial multiplication, the cells laid on the surface of agar may, from the beginning, exhibit a very low level of metabolic exchange. In consequence, changes of reaction and the possible production of inhibiting substances take place to a slight degree and slowly, and the products diffuse away from the cells into the agar.

Since heavy inoculation is necessary in order to obtain yields suitable for vaccine production—which is the ultimate purpose of the work—we have had to carry these cultures to the 5th agar generation and a dilution, in terms of tissue used—of the original Maitland culture of approximately 1 to 700—before we could be sure we were not dealing with organisms carried over.

We are at present engaged in improving methods of controlling the reaction and determining the suitability of embryonic tissue and other cellular material more easily procurable in considerable quantities than guinea pig tunica.

Although probably not in its final form, the method appears to us to offer hope of solving in a simple manner the problem of vaccine production for human protection against both the murine and the classical European varieties of typhus fever. It is not out of question that it may be applicable to virus cultivation as well since the cells are no doubt to some extent active for 48 hours.